GROWTH AND BIOCHEMISTRY OF THE COMMON HYACINTH

(*Hyacinthus orientalis* L.) AND THE LILY (*Lilium longiflorum* L.)

BY

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DECLARATION

I hereby declare that this thesis has not been previously submitted, either in the same form or different form, to this or any other University for a degree

Signed

Isaac Kwahene Addai

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ABSTRACT

The biochemical principles underlining carbohydrate metabolism of ornamental geophytes such as hyacinth and the lily are poorly understood. The present studies were therefore undertaken to investigate the regulation and partitioning of carbohydrates, as well as growth and development of these flower bulbs. Results indicated that starch was the major storage carbohydrate in these bulbs. Starch degradation occurred through amylolysis rather than phosphorolysis. The flower accumulated the highest amount of the reserves in hyacinth, following the depletion of these substances in the bulb scales, whilst in the case of the lily; it was the stem and roots, which accumulated the highest amount of reserves. The isolation of gene fragments of starch phosphorylase and starch synthase from hyacinth as well as the sequences generated for these enzymes implies that primers which are specific to hyacinth can be designed, and full characterisation of the genes can be made in the future by making and probing genomic libraries and isolating clones from cDNA libraries.

In general, peeling of bulbs prior to planting resulted in a delay in emergence and reductions in vegetative growth as well as flower quality. Hyacinth plants subjected to defoliation did not show any compensation for leaf loss because growth was always reduced whilst flowering was unaffected, however, the lily responded positively to complete shoots removal especially when herbivory occurred at the beginning of their growth. Plants produced from large bulbs, just like those from the shallow planting regimes performed better in terms of growth and flower quality as compared to those of small bulbs and deep planting. Also, the application of $(NH_4)_2SO_4$ enhanced more vigorous growth and bulb yield in both species of flower bulbs than did Na_2HPO_4, but in hyacinth it was Na_2HPO_4 that produced better flower quality than the former. Varieties such as Sky Jackets and Purple Voice produced the highest vegetative growth and bulb yield, whilst Fondant and Blue Jacket recorded the highest flower quality.

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LIST OF ABBREVIATIONS

ADP = adenosine diphosphate

AGPase = ADP-glucose pyrophosphorylase

ATP = adenosine triphosphate

BSA = bovine serum albumin

EDTA = ethylene diamine tetraacetic acid

ELISA = enzyme linked immunosorbent assay

Hepes = N- [2-Hydroxyethyl] piperazine-N^1- [2-ethanesulfonic acid]

NAD = nicotinamide adenine dinucleotide

NADH = reduced NAD

NADP = NAD phosphate

NADPH = reduced NADP

PEP= Phosphoenolpyruvic acid

SPAD = Soil Plant Analytical Division

3 PGA = 3- phosphoglycerate

PPi = pyrophosphate

Pi = inorganic phosphate

r = correlation coefficient

Tris = Tris [hydroxymethyl] aminomethane

PVP = polyvinylpyrrolidone

SDS = sodium dodecyl sulfate

TBE= Tri/Borate/EDTA

WAP/ MAP = weeks after planting/ months after planting

CHAPTER 1

GENERAL INTRODUCTION

1.1 The geophyte

A geophyte is a plant that has the ability to survive unfavourable environmental conditions by dying back to underground storage organs. A number of reserved carbohydrates such as starch, soluble sugars, glucomannans and fructans; proteins, mineral salts and water may be found in their storage organs (Miller *et al*., 1997). The geophyte uses these reserves to sprout when environmental conditions become favourable. In general, these storage organs are not physiologically dormant, even though there may be no aerial growth. The aerial parts of the plants die off when there is drought, or during the winter season and the underground part, which is the storage organ, is left buried in the soil. Propagation in most geophytes is by division or proliferation of the underground portion of the plant such as bulbs, rhizomes and the tubers.

According to Al-Tardeh *et al*. (2008), the evolution of geophytes in climatic areas with marked seasonal variations has resulted in their adaptation to periods of high or low temperature or drought. They are able to do this by exhibiting adaptations such as increased capacity for water binding, tolerance of, or resistance to desiccation and drought, and formation of subterranean organs (Kamenetsky, 2005). According to Bewley (2002), some of the storage proteins of geophytes may function as temporary sites for nitrogen and are mobilised later in the season to support the growth of the plant. Al-Tardeh *et al*. (2008) also stated that the order in which the different organs of geophytes are differentiated is very crucial. In situations whereby flowers and leaves appear at the same time, or when the foliage is produced before flowering (as in *Hyacinthus, Tulipa*, *Lilium* and *Narcissus*), the phenomenon is called synanthy, but sometimes the leaves expand after the flowers have opened (hysteranthous). Similarly, flowers of some geophytes appear before the leaves, or the foliage dies down before the flower is produced, as occurs in *Boophane haemanthides*, and this is termed proteranthous growth. According to De Hertogh and Le Nard (1993), the synanthous type of flowering occurs in late spring whilst the hysteranthous pattern occurs at the end of summer. These authors reported that in hysteranthous geophytes, the rate of photosynthesis is very low from flower emergence until anthesis because the reserves found in the storage organ is sufficient enough to allow flower stem elongation and flowering. However, in proteranthous geophytes, all photosynthesis takes place before flowering occurs. Corms, rhizomes, stem tubers, root tubers and bulbs are all geophytes.

A corm is a short, vertical, swollen underground plant stem with one or more internodes and at least one growing point, with protective leaves that is modified into skins or tunics. A rhizome is, however, a horizontal stem that often sends out roots and shoots from a node. A stem tuber may be formed from rhizomes or stolons; the top part may produce shoots that develop into stems and leaves whilst the under sides form roots. A root tuber is a modified lateral root that may enlarge to function as a storage organ.

1.2 The flowering bulb

A bulb is made up of a modified stem containing a complete miniature plant, including embryonic leaf, stem, and flower parts, and surrounded by fleshy scales which provide food for the young plant and a basal plate (Fig 1.1) which produces roots (National Gardening Association, 2005). Bulbs are also surrounded by a thin protective layer called tunic. Laminate (tunicate) bulbs are concentric, cylindrical scales. The outer layers of scales become dry and papery, forming the tunic. This protects the bulb from disease, insect and mechanical damage. Daffodil and onion are common tunicate bulbs. Two main types of this layer are known based on the protective covering. One, typified by onion, has a thin papery covering protecting its fleshy leaves. The other, the scaly bulb, as seen in true lilies, has naked storage leaves, with no papery covering, making the bulb appear to consist of angular scales. Most bulbs consist of a much-compressed, fleshy stem, the basal plate. Attached to the basal plate are thick, fleshy, modified leaves, the scales. The scaly (non-tunicate) bulbs have numerous individual scales which readily break off the basal plate. This type of bulb has no tunic, making it somewhat more susceptible to disease, insect and mechanical damage. Lily is an example of a common scaly bulb.

Most bulbs are made up of short stems (Fig 1.2 and 1.1) that bear a number of swollen fleshy leaf bases or scale leaves with or without a tunic, the whole enclosing the next year’s bud. Bulbs may have localised groups of cells that remain meristematic as found at the junction of the basal sheaths and the long flat blade of narcissus foliage leaves (Chen, 1960) and just below the flower in tulips (Sachs, 1962). Bulbs such as hyacinth possess adventitious buds that develop from epidermal and sub epidermal cells and not from pre-formed meristematic tissues as might be expected. There are no zones of cells which continue division indefinitely and no formation of additional vascular tissues

once the growth in width of the stem is completed. Some bulbs such as the tulips exhibit dropper formation (Stolon plus bulblets) in the seedling stage whilst in other bulbs like erythronium, the phenomenon occurs when the plant is already mature (Robertson, 1906).

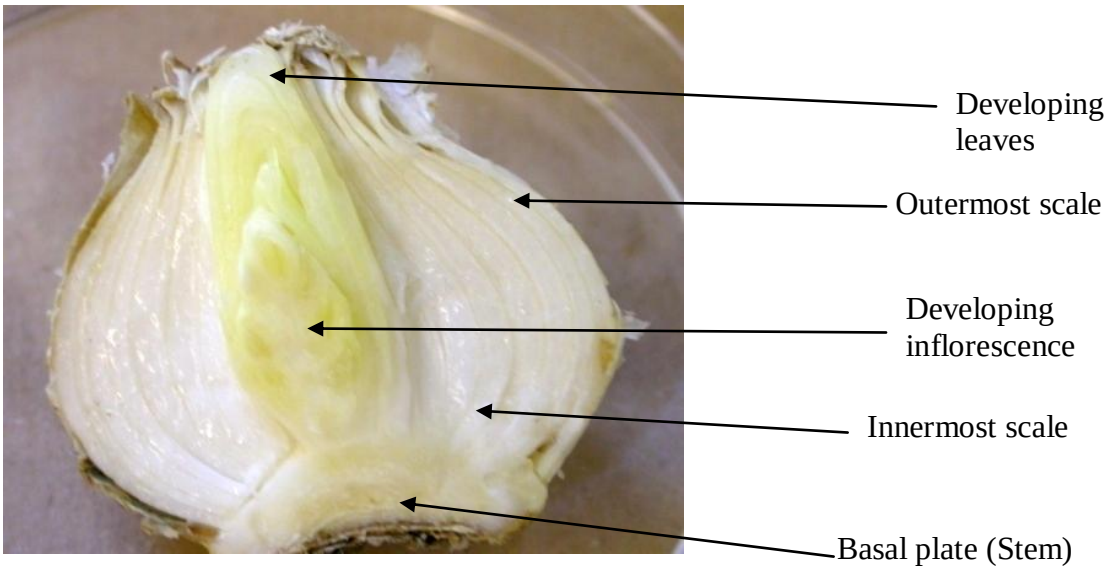


Fig 1.1: Longitudinal cross section of a hyacinth bulb

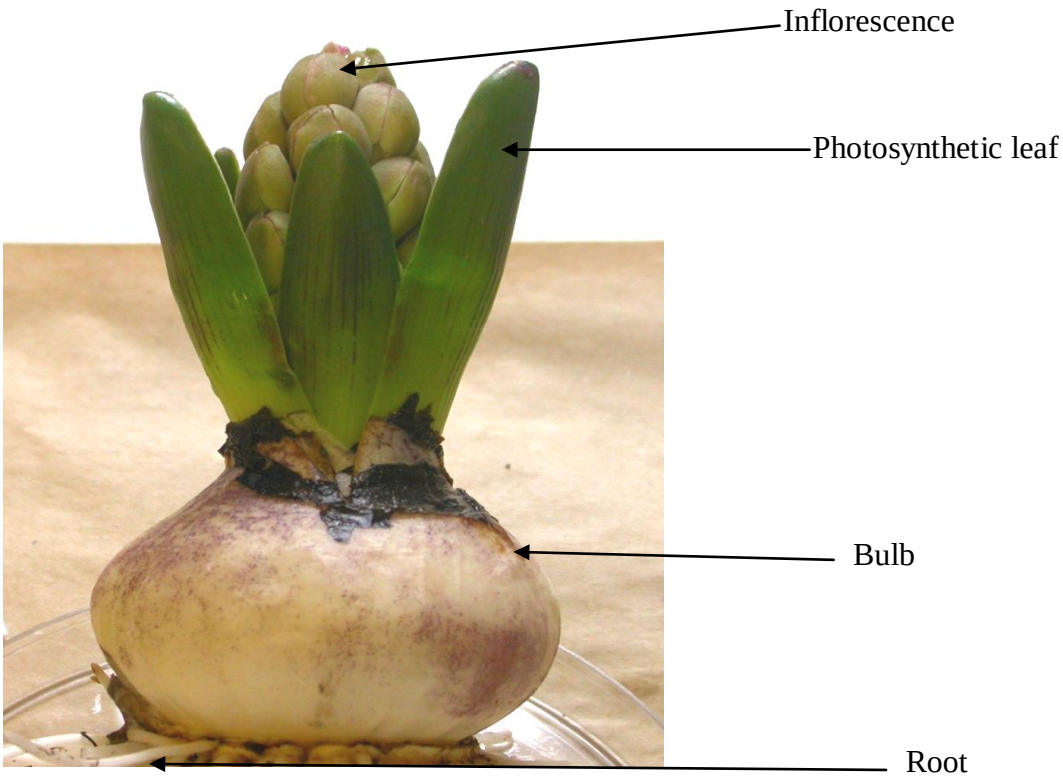


Fig 1.2: The external features of a hyacinth bulb, picture taken at 12 weeks after planting of the bulb.

Droppers are therefore a form of stolons where the extended portion is a continuation of a base of the foliage leaf, the morphology of which is partly foliar and partly axial. In some genera, there is the development of contractile roots that pull the bulb down into the soil (Wilson and Honey, 1966). Resting periods may occur between the death of shoots and the replacement by basal buds. This could perhaps occur by the death of the mother axis root system and the failure of the daughter bulbs to establish a new root system until soil moisture conditions improved. In order for this to occur, each new growth starts from an underground lateral bud, and that the underground should develop adequate storage tissues to enable survival during the unfavourable period. The replacement of aerial parts of limited height and duration by basal shoots tends to give clumped or tufted growth. The possession of summer dormancy and a cold requirement is good for the development of storage temperature treatments allowing controlled flowering. The dormant bulb, however, is a convenient stage for handling, transport and treatments.

There are some physiological considerations to regard in the growth of bulbs. For the developing plant, the possession of a bulb in some ways is equivalent to growing from a large seed. The bulb allows the formation of a large shoot before this is exposed to the outside environment, and the subsequent growth of the shoot and the root is independent of current photosynthesis. This means that growth early in the spring is ensured in temperate latitudes where incoming radiation is low. Food reserves of the flowering bulbs allow the plant to survive extended periods when growth is not possible, such as in cold winters and hot dry summers. These characteristic features of these plants may explain why most species are found in areas with Mediterranean climates. Bulb plants may be found in a wide range of habitats from tropical forests to open grasslands and deserts. They are mostly found growing between 23-45^{0}N and S latitudes. Burns (1946) reported that climate and day-length are responsible for the evolution of these geophytes. Savos'kin (1960) also stated that regular onset of a hot dry climate is of importance in the evolution of flowering bulbs, and that the development pattern is caused by the Mediterranean climatic rhythm of warm, wet winters and hot dry summers.

One important physiological feature of flowering bulbs is also their mode of propagation. Daughter bulbs are produced generally in the axils of their scales, foliage

leaf bases or in other positions and these are responsible for the occurrence of clumps of plants derived from the same parent bulb, although these clumps are seen more commonly in temperate gardens than in their natural habitat. Thus many bulbs reproduce via bulblets also referred to as offsets. These bulblets formed on the 'mother' bulb are sometimes also termed baby bulbs and they grow over time. Once they have accumulated enough energy they can be separated from the mother bulb to grow new plants. However, when pollinated, flowers of most bulbs produce seeds, and these seeds can also be used to grow new plants.

1.2.1 Spring and summer-flowering bulbs

Bulbs are divided into two groups based on their blooming time: Spring-flowering bulbs and Summer-flowering bulbs. Spring-flowering bulbs such as daffodils, crocus, tulips and hyacinth are planted in the fall for spring bloom. They are also called hardy bulbs because they survive cold winter conditions and they need exposure to cold temperatures in order to flower properly **(**National Gardening Association, 2005)**.** Blooming throughout the spring months, they produce bright, cheery, and often fragrant flowers that herald the return of warmer weather. The exact timing for planting hardy bulbs varies by region, but they need to be in the ground before it freezes. Proper planting time is important not only for winter survival, but to insure adequate root development, which results in better flower production. Roots are formed soon after planting, and then they lie dormant during much of the winter. When the weather begins to warm, they utilize the stored energy to produce flowers and leaves. Some members in this group, such as crocus, require fewer cold hours and less warming to bloom, so their flowers emerge in early spring. Others, such as tulips, need a longer cooling period and warmer temperatures to emerge, so that they can bloom later in the spring **(**National Gardening Association, 2005). Differences in blooming times allow planning a bulb garden that will bloom for a desired number of weeks. If chilling is enough, hardy bulbs usually sprout leaves when the weather warms, but if they bloom at all, the flower stalks will likely be stunted. Bulbs, like all other plants absorb nutrients from the soil and manufacture their food through photosynthesis. Assimilates not used for their daily living is stored in their storage organs for next year's growth. Once there is enough energy in these organs, leaves turn brown and die. The bulb enters a dormant state

through the summer, autumn, and winter months until it is time to sprout out again the following spring.

Summer-flowering bulbs, including dahlias, begonias, lilies and gladiolus are planted in the spring for summer bloom. They are tender and may not survive when the temperature is very low resulting in extremely cold winter conditions. However, there are summer-flowering lilies that are hardy enough to survive winters in some areas. After planting these bulbs, they grow much the same way as spring-flowering bulbs, and produce and store food for next year. A number of bulbs in this category may keep their leaves and continue to photosynthesize until autumn temperatures turn cool. It is a common routine for people in northern climates to dig up tender bulbs and store them in a dark place that stays cool enough to keep them from sprouting prematurely. However, where winters are warm enough, tender bulbs could be left right in the ground but it is important that the hardiness zone of the bulbs are known so as to determine their proper care.

Some flower bulbs are cultivated in their native habitat condition as found in tropical and sub-tropical countries, or under artificial conditions in greenhouses, but information on their adaptation to different conditions of temperature, humidity and light are important. Many bulb species are cultivated as ornamentals and this is the chief economic value of the plant. In general, flowering bulbs are an important addition to any landscape or garden. The great variety of bloom colour, flowering time, plant height and shape makes bulbs a good addition to the landscape. Over 160 genera of *Liliaceae* are represented in American trade. Hyacinth types, lily, meadow saffron, squill, and tulip constitute the bulk of the "Dutch bulb" trade. Asparagus and plants of the onion genus are liliaceous food plants of commercial importance.

1.2.2 Some physiological disorders, diseases and pests of bulbous plants

Bulbous plants have a number of disorders, diseases and pests. Most of the disorders of these plants, generally, have no known causal organisms but their effects are attributable to unfavourable environmental conditions such as frost, hail, water logging, water stress or the failure of the grower to follow certain guidelines necessary for their growth and development. Bulbs disorders may give rise to a complete failure of flowering or

rooting (Rees, 1972). For instance, the loose-bud disorder of the common hyacinth also termed *spouwen* or spewing, leads to the ejection of the inflorescence shortly after flowering. The inflorescence gets separated from the peduncle and is carried up by the leaves which become opened such that the inflorescence is pushed to one side. This disorder mostly attacks outdoor-planted plants of hyacinth and is common with those that have been forced. Cultivars of hyacinth that are easily attacked include 'Pink Pearl' and 'First Bismarck'. According to Beijer (1947), loose-bud of hyacinth results from a sap infiltration of the base plate and the peduncle producing a water-soaked appearance. In severe cases, there is the formation of a narrow longitudinal cavity in the peduncle. Extension growth of leaves may carry the inflorescence upwards; the inflorescence then becomes loose and turgid as the lower ends are surrounded by sap that fills the cavity between the ends of the peduncle. Normally, a disturbance of equilibrium between water uptake by the roots and transpiration from the shoots may result in this condition. Other factors that may lead to this condition include lack of high temperature during summer treatment of bulbs, too early planting of the bulbs, too high soil temperature after planting, too high soil temperature during winter that will also lead to water and solute absorption, too much water available to plants during the growth of plants and too much use of ammonium sulphate fertilizer which affects cell permeability. Beijer (1963) stated that mechanically, loose-bud of hyacinth occurs because the peduncle may be broken at its base and no short stump is left attached to the base plate and therefore air fills up the resultant cavity. This occurs when plants are moved from one location to another as for example, moving plants from greenhouse to outdoors or vice versa. Similarly, the condition occurs when temperature is fairly constant or is increased gradually. Another physiological disorder of hyacinth is root failure. According to Moore (1939), this abnormality occurs when early shoot is normal and no roots are either formed or only a few develop but die within a short time. This often leads to failure of the shoot to grow, and wilting or necrosis may occur. Root failure becomes rampant in cold summers but the condition is worsened by too early forcing or lifting of the bulbs. Another disorder of hyacinth worthy of mentioning is forcing failures. It occurs when bulbs are incorrectly stored and this leads to flower abnormalities such as death of some flowers on the inflorescence before opening or a complete failure of the whole inflorescence. Gummosis, a situation whereby the bulb produces excessive gum, has been reported in hyacinth. The condition is caused by the fungus *Fusarium*. In this condition, ethylene is produced and gets accumulated on or near the bulb in the soil.

The yellow disease of hyacinth is caused by *Xanthomonas hyacinthi*. This disease normally causes the bulb to rot before or soon after planting and shoots do not emerge after planting or the shoot may emerge but no inflorescence is formed. When a transverse section is cut, yellow spots arranged concentrically are revealed. The spots are longitudinal bands that reach the base plate from where they could also infect healthy scales. The disease may spread quickly especially when there is a cut on the leaf, or on the peduncles particularly when an inoculum from infected plants reaches there by wind or rain splash. High storage temperatures such as 30 ^{o}C are suitable for the transmission of this disease. Prevention of the disease is by close scrutiny in the field to find infected plants and destroying them. Bacterial soft, caused by *Bacterium carotovorum* occurs at flowering. Usually, the tips of leaves become yellow and the tips shrivel and dry out such that growth of plants normally stops. The scape rots near the ground level, the inflorescence falls over and usually the leaves and the whole bulb rot. Control of this disease is by removal and destruction of the infected plants and sterilization of soil prior to planting. Hyacinth root rot, caused by *Pythium spp* (e.g. *P. ultimum* and *P. Violae*) also often leads to loss of leaf turgor and tip death. The disease is controlled by disinfecting the suspected soil using formalin or methamsodium before planting. Also hyacinth foliage is normally attacked by *Botrytis hyacinthi*. The disease is often referred to as 'fire' and usually occurs during damp springs and leads to destruction of the leaves and flowers. The disease is controlled by spraying of fungicidal sprays.

Abnormalities that may affect the lily plant include flower bud abortion, which occurs at a stage in flower development and this is called 'bud blasting'. The condition is characterised by the base of the flower bud becoming light greenish and then yellow. The bud then shrivels and turns brown. The disorder usually attacks the cultivar 'Georgia' and is rampant in south-eastern parts of United States. Einert and Box (1967) found a correlation between this condition and flower number per inflorescence and concluded that competition for nutrients is paramount to this abnormality and Smith and Langhans (1961) also reported that drought may play a role in aggravating this abnormality leading to flower abortion. Other factors such as increased soil nitrate level (Eastwood, 1952) and reduction of carbohydrate supply to the developing flower buds through leaf herbivory (Mastalerz (1965) also increase flower bud abortion. Lily suffers from Botrytis blight, caused by *Botrytis elliptica*. This disease is a threat to field-grown

lilies (Dimock and Tammen, 1967) and is characterised by small reddish-brown spots on leaves that often spread to attack the pedicel and flower buds killing the foliage. Transmission is aided by moist cool weather and fogs. Sanitation and routine spray programme are necessary to control this disease. The lily bulb also suffers from root-rot complex caused by *Rhizoctonia* and *Pythium spp*. It is characterised by browning and rotting of roots and death of the whole plant. The control measures involve hygiene, sterilisation and use of fungicides.

1.2.3 Family *Hyacinthaceae*

Most members of the family *Hyacinthaceae* were initially considered to be part of family *Liliaceae. Hyacinthaceae* is known to comprise about 500-700 species of bulbous or rhizomatous herbs in 41 genera. Plants in this family are usually found in the Mediterranean region and in South Africa, and are cultivated as ornamental plants (Mabberley 1997). Plants in the family *Hyacinthaceae* generally have skin irritant properties because of calcium oxalate needle crystals they contain. In some species, mechanical injury to the skin may be worsened by the irritation of chemicals in the plant sap. Dermatologically, plants in this family may be used to prepare a lotion for treating sore eyes and boils. Watson and Dallwitz (1992) described members of *Hyacinthaceae*. Plants generally have alternate or spirally arranged leaves with leaf sheaths that have free margins. Leaves can be simple and are linear, or lanceolate, and they have parallel-venation. Stomata are usually present on these leaves and the mesophyll contains mucilage cells usually with raphides. The stems do not have secondary thickening and their tissues have no vessels. The root xylem rather has vessels and their end walls are scalariform and simple in nature. Plants are generally hermaphroditic, and nectar secrete from the gynoecium. The flowers aggregate to form inflorescences. These flowers are terminal and are usually simple or form branched racemes. There are six-free or joined perianth of petals, two of which may be whorled. Their colour could be white, yellow, red, violet, blue, brown, or black. The stamen is six in number or three as in *Albuca* where the outer whorl may be reduced or absent. The filaments are appendiculate or sometimes appendaged by lobes on either side of the anthers which are dorsifixed and dehisce through longitudinal slits. The *gynoecium*, which could either be stylate or non-stylate, is made up of three carpels.

1.2.3.1 The common hyacinth

The common hyacinth, *Hyacinthus orientalis* belongs to the family *Hyacinthaceae* and genus *Hyacinthus*. It is a horticulturally important plant, and native of the West and Central Asia. The diploid forms of hyacinth plant have 16 chromosomes of 5 different types and there are also triploids (3n = 24) and a large number of heteroploid (Rees, 1972). Several species are cultivated, but the most well known is *Hyacinthus orientalis* L. This species was first developed by artificial selection in Turkey and subsequently in Holland (Gorer, 1970). The most commonly encountered cultivars of this species as well as their characteristic features are shown in Fig 1.3. The mature hyacinth bulb, at lifting has a dead inflorescence stalk surrounded by the bases of foliage leaves whose aerial parts have recently died (Blaauw, 1920). Surrounding these are the bases of the foliage leaves of the previous year and outside these again are the two scale leaves of the corresponding annual cycle. The leaf bases and scales are usually fleshy and white, and are swollen with food reserves. Older leaf bases and scales, which have little reserved food, again surround the previous scales and leaf bases. The outermost scales are thin and papery. *Hyacinthus orientalis* is a spring flowering bulb. Flowers are hermaphroditic, and are generally pollinated by bees (Davis, 1990); they have a strong sweet fragrance that can fill the air for a considerable distance (Gender**,** 1994). The leaves are deep green, strap-like and are formed in clumps. Hyacinths generally grow between 10 and 30 cm tall and come in a wide range of colours (Fig 1.3). Flowers are usually between 15 and 20 cm tall. The swollen portion consists mostly of fleshy, food-storing scales attached to a short flat stem. The plant prefers moist but light sandy to loamy soils. These soils could be acidic, neutral or alkaline. The dormant bulbs are fairly hardy, and could withstand soil temperatures down to at least -5°C (Matthews, 1994). De Hertogh and Le Nard (1993), however, reported that hyacinth requires a cold period of -0.5 to 10 ^{o}C for 10-18 weeks for optimal shoot elongation in spring. Banaski *et al*. (1980) also reported that appropriate sequence of warm and low temperature is important for normal development of the flower and its emergence.

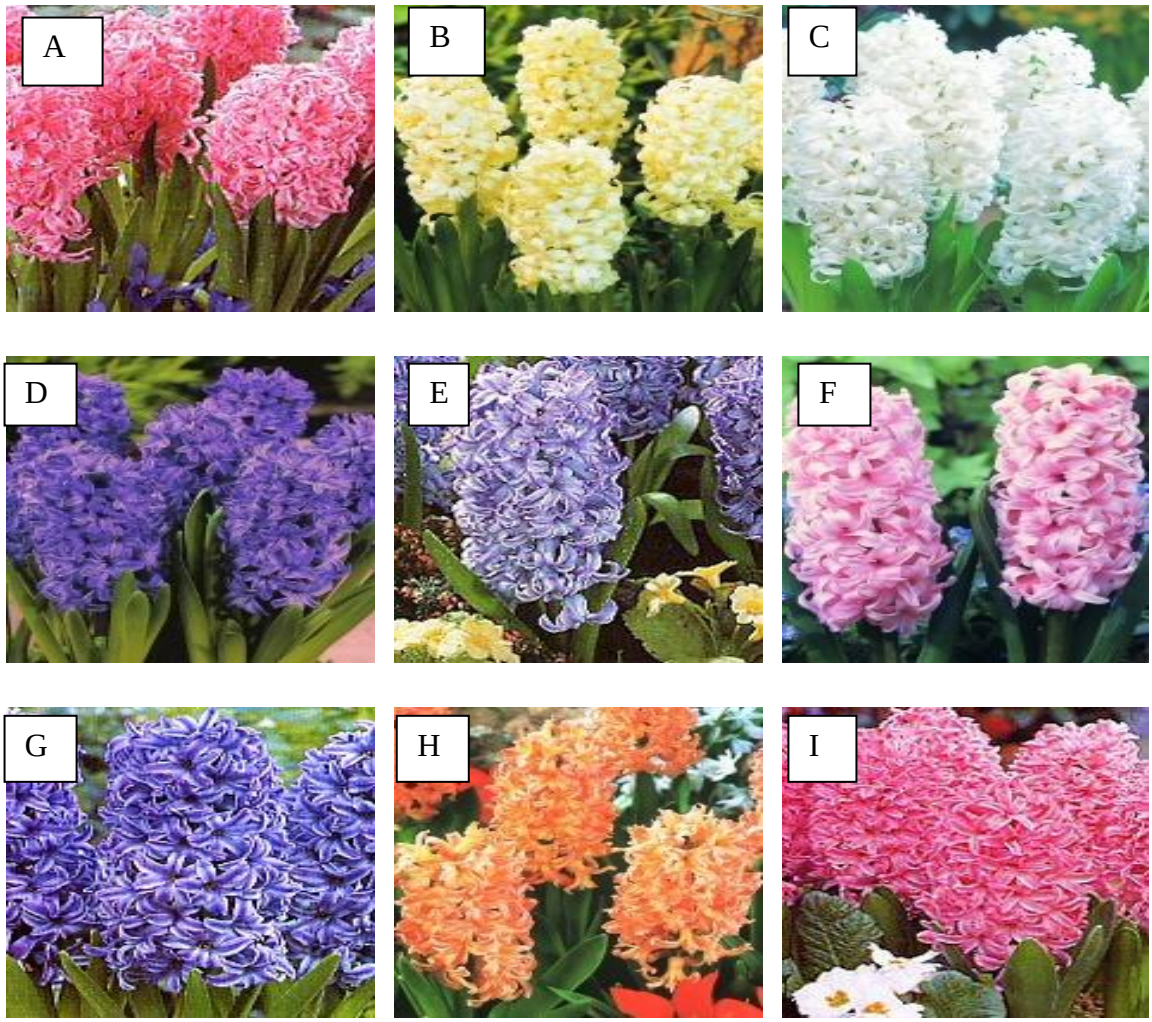


Fig 1.3: Characteristics of some cultivars of the common hyacinth.

Ann Mary (A) is sweetly scented bells of old rose becoming salmon with age. It has neat head of medium size and its compact habit makes it ideal for tubs and window boxes. **City Haarlem** (B) is 25 cm in height. It has primrose yellow bells placed closely on a long elegant spike. **Carnegie** (C) is 25 cm in height. It has a serenely lovely flower; purest white large fleshy bells making up a broad spike. **Atlantic** (D) is 20 cm in height. It is famous for its superb blending of all beautiful colours. **Delft Blue** (E) has a height of 25 cm. It is one of the earliest of the blues. The bells are porcelain blue, lighter in the centre and the outside is a soft mauve. **Splendid Cornelia** (F) is 20 cm in height. It is an enchanting hyacinth with delicate lilac mauve bells turning silvery lilac with age. **Blue jacket** (G) is 25 cm in height. It produces a solid spike of large bluebell flowers silvering slightly towards the edges of the long petals. **Gipsy Queen** (H) is 20 cm in height. It has a lovely shade of Chinese yellow heavily flushed apricot tangerine and strong trusses of closely set bells of a beautiful colour. **Pink Pearl** (I) is 25 cm. It has a large solid spike with substantial bells of clear rose pink silvering with age towards the edges.

1.2.4 Family *Liliaceae*

The Lily bulb belongs to the family *Liliaceae*; order *Liliales* and the genus is *Lilium*. Plants belonging to family *Liliaceae* are also important ornamental or houseplants that are grown for their attractive flowers. According to Liang Song-yun (1995), the Irano-Turanian region has many species of this group of plants but the Eastern-Asian region is the centre of diversity of *Liliaceae*. The author believed that *Lloydia, Erythronium, Fritillaria and Lilium* originated from the North temperate region, whilst *Gagea* and *tulipia* are assicated with the Old World temperate region. The West Asia to Himalayas and South West China are rich in *Notholirion*, whilst *Cardiocrinum* and *Nomocharis* are natives of East Asia. Afrozi and Hassan (2008) described the family *Liliaceae*. In general *Liliaceae* comprises perennial plants that have starchy rhizomes, corms, or bulbs. *Liliaceae* is also made up of about 280 genera and 4,000 species. Leaves of plants from this family are alternate or less often opposite or whorled. The flowers are often showy, bisexual and actinomorphic. The perianth may consist of two whorls of undifferentiated or nearly differentiated petaloid tepals with three distinct members in each whorl. The androecium may consist of six fertile stamens attached to the receptacle. The gynoecium, however, is made up of a single compound pistil of three carpels, a single style commonly with three stigmas, and a superior ovary with three locules, each containing several to numerous axile ovules.

1.2.4.1 The lily

The genus *Lilium* includes the true lilies. They are native primarily to temperate and subtropical regions. The true lilies are erect perennials with leafy stems. They produce scaly bulbs and narrow leaves, and solitary or clustered flowers of which some are quite fragrant, with a variety of colours. Most species store nutrients underground in a bulb, corm or tuber. They grow wild throughout Europe, northern Asia and North America. Their range in the Old World extends across much of Europe, the north Mediterranean, across most of Asia to Japan, south to the Nilgiri Mountains in India, and south to the Philippines. In the New World, lilies extend from southern Canada through much of the United States. They are commonly adapted to either woodland habitats, often montane, or sometimes to grassland habitats. A few can thrive in marshland, and some such as *L. arboricola* is known to exist as an epiphyte. In general, lilies prefer moderately acidic or

lime-free soils. The lily bulb is covered with densely-crowded spirally-arranged thick scale leaves which are not concentric as in many other bulbs (Rees, 1972). Leaves of two kinds are produced: the first appear in the autumn at the base of the rudimentary flower stem of the following year, the other leaves are borne on the emergent, tall, flowering stem in summer. The radical leaves die down, but their bases remain as scales, each of which has a blunt tip where the emergent blade was attached. Within these are true scales which have pointed tips, and within these again are the radical leaves of the current season, whose bases later become swollen. In the axil of the innermost radical leaf, at the base of the young flowering stem is a tiny daughter bulb which becomes the next season's bulb and shoot. The leaves are lanceolate, without petioles, but with bases that embrace about a third of the stem's circumference. The flowering stem does not branch, however, buds occur in the axils of the leaves and these may develop into aerial bulbs that can be used for propagation, but the extent to which this is expressed is reliant on the species and variety. Blaney and Roberts (1966) investigated into growth and development of the Easter lily, and concluded that the bulb is concentric with closely imbricated scales each of which bears an axillary bud. On the lower surface, basal roots are borne; stem roots also occur on the 'below-soil part' of the stem above the bulb. Small bulbs, called stem bulblets, could be found in the underground part of the flower stem. With these, the bulb grows naturally at some depth in the soil, and each year the new stem develops adventitious roots above the bulb as it emerges from the soil. Flowers are formed at the top of a single erect stem, with leaves being borne at intervals up the stem. The large flowers have three petals along with three petal-like sepals, often fragrant, and come in a range of colours: white, yellow, orange, pink, and red, purple, bronze and even nearly black.

The true lilies are propagated in four main ways: through a division of the bulbs, by use of bulblets, by detaching scales from the bulb and planting to form a new bulb and by the seed. Many species of *Lilium* are generally planted in the garden in temperate and sub-tropical regions as potted plants (Miller and Kofranek, 1966). Many hybrids from this group of plants have been developed and they are used in herbaceous borders, woodland and shrub plantings, and as a patio plant. Some lilies, especially *Lilium longiflorum*, as well as a few other hybrids, are important cut flower crops and they are forced for particular markets. As for instance, *L. longiflorum* for the Easter trade, when it is called 'the Easter lily'. Bulbs of *Lilium* species are starchy, and some are edible as

root vegetables. However, bulbs of a few other species may be very bitter. The non-bitter bulbs of *L. lancifolium*, *L. pumilum*, and especially *L. brownii* are commercially grown in China as a luxury or health food, and are generally sold in dry form. They are eaten especially in the summer, because they have the ability to reduce internal heat. They may be reconstituted and stir-fried, grated and used to thicken soup, or processed to extract starch. Their texture and taste are comparable to potato, though the individual bulb scales are smaller. Even though they are believed to be safe for humans to eat, there are evidence of nephrotoxicosis that is kidney failure in cats which have eaten some species of *Lilium* and *Hemerocallis* (Chittendon, 1956; Huxley, 1992). Bulbs of Tiger Lily, *Lilium lancifolium* Thunb, may be cooked and eaten (Hedrick, 1972; Fox, 1985; Grieve, 1984) but it is somewhat bitter (Tanaka, 1976), however, when properly cooked, they are highly esteemed as a vegetable. The cooked bulbs resemble parsnips in flavour (Facciola, 1990). The bulbs are a good source of starch (Tanaka, 1976; Facciola, 1990) and can be dried and ground into powder. Flowers are used fresh or dried in salads, soups and rice dishes (Facciola, 1990). According to Chopra *et al.* (1986) and reports by World Health Organisation (1998), the bulb is anti-inflammatory and diuretic. However, these bulbs are used to relieve heart diseases, pain in the cardiac region and angina pectoris (Chopra *et al*., 1986). They are also used in Korea to treat coughs, sore throats, palpitations and boils (World Health Organisation, 1998). The flowers are carminative (Chopra *et al*., 1986) and can be used to strengthen the eye-lid muscles, and also in the treatment of myopic astigmatism (Chopra *et al*., 1986). A tincture made from the flowering plant, harvested when in full flower, is also used to cure uterine neuralgia, congestion, irritation and the nausea of pregnancy (Grieve, 1984). It relieves the bearing-down pain accompanying uterine prolapse and is an important remedy in ovarian neuralgia (Grieve, 1984). Lilies also generally serve as food plants for the larvae of some Lepidoptera species including the Dun-bar. Different forms of lilies are grown for the garden, and most of these are hybrids but they may differ according to their parent species. The artificial grouping accepted by the Royal Horticultural Society and the North American Lily Society (See Fig 1.4) is used in classifying the plant.

Fig 1.4: Classification of the lily flower bulb.

Longiflorum hybrids (A) are cultivated forms of this species and its subspecies. They are most important as plants for cut flowers, and are less often grown in the garden than other hybrids. **Asiatic hybrids** (B) are plants with medium sized, upright or outward facing flowers, mostly unscented. They are derived from central and East Asian species. **Trumpet lilies** (C) include Aurelian hybrids. This group includes hybrids of many Asiatic species, including *L. regale* and *L. aurelianse*. The flowers are trumpet shaped, facing outward or somewhat downward, and tend to be strongly fragrant, often especially night-fragrant. **Oriental hybrids** (D) are based on hybrids of *L. auratum* and *L. speciosum*, together with crossbreeds from several mainland Asiatic species. They are fragrant, and the flowers tend to be outward facing. Plants tend to be tall, and the flowers may be quite large. **American hybrids** (E) are mostly taller growing forms, originally derived from *L. pardalinum*. Many are clump-forming perennials with rhizomatous rootstocks. **Species** (F), all natural species and naturally occurring forms are included in this group. **Oriental-trumpet hybrid** (G), they share the properties of Oriental and trumpet lilies. **Martagon hybrids** (H) are based on *L. martagon* and *L. hansonii*, flowers are nodding, and petals strongly recurved. **Candidium hybrids** (I) includes hybrids of *L. candidum* with several other mostly European species.

1.3 Carbohydrate partitioning and metabolism

According to Miller (1992), starch is the major storage carbohydrate in most plants, and is nearly ubiquitous throughout the plant kingdom. In plants, starch may exist either as unbranched amylose, with chains of about 1000 glucose residues (α-1, 4-linked D-glucose residues), or as amylopectin, a branched polymer with perhaps 25–50 glucose residues between α–1, 6-branch points. In general, starch synthesis in plants is known to be regulated by the enzyme ADPglucose pyrophosphorylase (ATP: a-glucose- 1-P ad-enylyltransferase, EC 2.7.7.27). This enzyme catalyzes the synthesis of ADPglucose which is the substrate for starch synthase (ADPglucose, 1, 4- a-D-glucan 4-a-glucosyl transferase, EC 2.4.1.21). ADPglucose pyrophosphorylase from most tubers and endosperm (Dickinson and Preiss, 1969) and leaves of C-3 and CAM plants (Sanwal *et al*., 1968) are activated by 3PGA but inhibited by Pi. Thus according to these authors, in plants, the rate of ADPglucose synthesis is controlled by the stromal 3PGA/Pi ratio, and this couples starch production to the rate of photosynthesis (Preiss, 1982). Preiss and Levi (1980) also stated that starch is synthesised in plants not only by ADPglucose pyrophosphorylase, but also through a series of reactions that involve ADPglucose pyrophosphorylase (EC 2.7.7.21), starch synthase (EC 2.4.1.21) and starch branching enzyme (EC 2.4.1.18). It is also known that, the carbohydrates stored in the underground parts of plants particularly most geophytes get mobilized during resprouting, and therefore act as the major supply of carbon for regrowth at the early stages after destruction to the above-ground part (Bowen and Pate, 1993). Bulbs, corms, tubers and other organs of plants contain a range of starch hydrolytic enzymes such as *β*-amylase (1,4-*α*-d-glucan maltohydrolase) [EC 3.2.1.2], which is an exoamylase that breaks down *α*-1,4-glucosidic linkages from the nonreducing ends of starch molecules releasing maltose and producing *β*-limit dextrin (Thomas *et al*. 1971); *α*-amylase (1,4-*α*-d-glucan glucanohydrolase) [EC 3.2.1.1], an endoamylase responsible for degrading the nonterminal glucosidic linkages to produce glucose, maltose, maltotriose, and branched oligosaccharides; *α*-glucosidase (*α* d-glucoside glucohydrolase) [EC 3.2.1.20], which also breaks down maltose and other oligosaccharides to glucose. Starch phosphorylase (1, 4-*α*-d-glucan; orthophosphate *α*-dglucosyltransferase) [EC 2.4.1.1] also hydrolyses starch leading to the formation of glucose-1-phosphate from starch and inorganic phosphate. When plants are damaged, there is mobilization of stored reserves in roots and other storage organs to support the growth of the above-ground tissue (Canadell and L´opez-Soria 1998). Miller (1992) reported that in most geophytes, the concentration of

starch and other carbohydrates may differ from species to species and from tissues to tissues. Similarly, the carbohydrate content of a plant at a particular time also depends on environmental conditions and may vary from time to time. Thus for example, during the early shoot growth of most geophytes, when stored reserves are utilized, starch content of the storage organs is expected to decrease, and subsequently increase after anthesis, because at this point, carbohydrate filling is rapid. According to Ohyama *et al*. (1998), starch was the predominant reserve carbohydrate in the mother bulb scales of tulips at the time of planting. The starch content, however, decreased continuously but was interrupted for two months until sprouting occurred and thereafter rapid starch consumption resumed so that at the time of anthesis, starch was depleted. These authors concluded that low temperatures were essential for the mobilisation of reserves, mainly starch, and the accumulation of the soluble constituents in the bulb scales and that when the solutes are transported into the shoot, they could be used for elongation and growth. In a similar study, they observed that dry weight of the mother bulb scales of tulip was found to decrease gradually after planting to half of the original value at the time of sprouting. The dry weight decreased rapidly until anthesis. Similarly, Ohyama *et al*. (1998) revealed that the total carbohydrates of the bulb of this plant were constantly consumed and only 44 % of the reserved carbohydrates remained in the bulb scales at the time of sprouting. The starch content in particular decreased continuously during the first three months and its rate of disappearance reduced during the fourth and fifth months and by the end of the six month after planting, starch content of the mother scales was almost completely depleted. Nowak *et al.* (1974) also reported that when both rooted and unrooted geophytes were exposed to a period of low temperature, the amount of carbohydrate initially presents in the scales, and bud from both categories decreased, though the decrease was greater in rooted than the unrooted bulbs. Levels of soluble sugars also increased in the scales and buds of the chilled bulbs. These changes in sugar levels were found to correlate with changes in the activities of alpha amylase and other hydrolytic enzymes. However, the activities of these enzymes were significantly higher in rooted than unrooted plants, and in flower buds than the bulb scales. In a related study, Miller and Langhans (1989) reported that differences occurred in the rate and capacity of depletion in bulb scales. They concluded that whilst the outer scales were essentially depleted of dry weight at anthesis, the inner scales were depleted in inverse proportion to the amount of light the plants received. Chen (1969), however, stated that in *Narcissus*, increasing irradiance had little effect on leaf starch levels.

Theron and Jacobs (1996) also studied the carbohydrate composition of different components of *Nerine bowdenii* bulbs and concluded that the developing inflorescence had a significant effect on source–sink relationships. Removal of bulb scales during active bulb filling stage resulting in loss of the major sink caused stems to accumulate over 4-fold more starch than the control stems (Wang and Breen, 1986). Also after studying the status of carbohydrate and water content of tulip bulbs, Lambrechts *et al*. (1994) observed that the dry weights, starch and soluble sugars of the scale leaves decreased some weeks after planting. In the same manner, Vishnevetsky *et al*. (2000) determined bulb fresh and dry weights, carbohydrate contents and the activities of enzymes related to carbohydrate metabolism in *Nerine sarniensis* cv Salmon at different stages of the bulb development, and concluded that starch was the dominant storage carbohydrate in these bulbs, and the leaf bases parenchyma cells were the principal storage tissue. During the first month of bulb growth, only small changes in starch content were detected but an increase in starch level was observed at later stages of development. Also, the activity of ADP-glucose pyrophosphorylase, a key enzyme of starch synthesis, increased just before the increase in starch accumulation. Sucrose was the dominant soluble sugar in the bulbs but only traces of glucose and fructose were detected. The activity of alkaline invertase was higher than that of acid invertase during the growth period. Sucrose synthase showed the highest sucrose degrading activity during bulb growth. Miller (1992) observed that stems of lily accumulate significant levels of starch, especially when normal source-sink relationships are altered. Generally sucrose serves as a primary transport carbohydrate in most plants and tissues of almost all geophytes contain sucrose and the common reducing sugars (glucose and fructose). Miller (1992) also reported that the concentration of sucrose in *Lilium longiflorum* increased during bulb reserve mobilisation and assimilates export but Miller and Niu (1990) observed that this carbohydrate showed significant diurnal changes in leaves, and the sucrose hydrolysing enzyme invertase was active in the flower buds. Thus the breakdown and metabolism of this sugar is of paramount importance since it facilitates growth of the storage organs, inflorescence and other sinks.

1.4 Response of plants to herbivory

Herbivory is an association whereby one organism called herbivore feeds on a plant or a plant-like organism. It determines the population abundance and dynamics of individual

plant species because herbivores may kill a whole plant, or affect its reproduction. Thus herbivores generally may regulate the species composition of plant communities or the total amount of plant biomass in the ecosystems. In plant-herbivore associations, growth of plants may be limited by factors such as the intensity by which the herbivore consumes the plant species and the availability of resources such as nutrients, water, and sunlight present in that area. Moreover, herbivory, help maintain species diversity in communities by removing plant species that compete intensively for resources, which allows other competitively inferior species to coexist (Burkepile and Duffy, 2009). The specific effects of herbivory on some plants species have been reported in literature. In the common hyacinth and lily, however, such information is either non-existent or very limited. As for example, Ruiz, Ward and Saltz (2002) removed 0, 25, 50 and 75% of bulb tissues from *Pancratium sickenbergeri* and observed that bulbs with the intermediate volume removed (50% peeling) showed the highest regrowth capacity and fitness in relation to the other cutting treatments. The control plants grew less, stored more energy, and produced more inflorescence and fruits than the other peeling treatments. Plants produced from the 75 % peeling treatments produced the lowest number of leaves. Peeling also reduced the ability of the plants to produce flowers. Plants from the unpeeled treatments (0% peeling) produced the highest number of inflorescence whilst those from the 75% peeling gave the lowest number of inflorescence. Number of fruits formed followed a similar trend. Similarly, leaf area and bulb dry weight reduced significantly in the peeled bulbs compared with the unpeeled control (Rees, 1971; Kim *et al.*, 2003). However, some plants have the ability to withstand and survive the damage caused by herbivory. They may achieve this by tolerance, induced defense or by compensatory growth. According to Karban and Baldwin (1997), induced responses by plants to herbivory may be referred to as induced defense if they would decrease rates of herbivory. Gadd, Young and Palmer (2001) reported a compensatory regrowth in *Accacia drepanolobium* in Kenya after studying the effects of simulated shoot and leaf herbivory on vegetative growth in that plant. Studies conducted by Rockwood (1973) and Marquis (1984) also revealed that heavy defoliation caused a depression in reproduction whilst Paige and Whitham (1978) maintained that under certain conditions, herbivory could stimulate reproduction but, it is generally known that, after defoliation, plants may use the reserves stored in their tissues for regrowth of vegetation (Rockwood and Lobstein, 1994), and this normally depletes the stored reserves, and negatively affects reproduction. Chapin *et al*. (1990),

however, found no exact correlation between stored carbohydrates and regrowth after defoliation. They attributed the reduction in reproduction to browsing that depletes the stored reserves. Lubbers and Lechwicz (1989), and Smith *et al*. (1986) also reported that leaf damage may not immediately influence reproduction but that future fitness could be affected but Primack and Hall (1990) concluded that with increasing severity of defoliation, reproduction may be reduced. Kalin (1954) also investigated into the effects of flower removal on bulb production in Narcissus and observed that the mean lifted bulb weight was highest following the deheading (removal of only flower heads) treatment whilst flower harvesting, even when done more carefully than in commercial practice, reduced bulb yield compared to the control. Further work also showed that the removal of the flowers as soon as the peduncle had grown long enough to enable this to be done was marginally better than deheading (Kalin, 1956). The general conclusion was that the peduncle was an important site for photosynthesis which would be retained for most efficient bulb production, and that deheading improves bulb yield. Banaski and Saniewski (1979) and Saniewski (1989) also observed that the removal of the leaves and flower buds completely inhibited flower stalk elongation. Endan *et al*. (2006) also observed that defoliation resulted in some reserved food material in the stems being redirected to the shoot region for new growth. Similarly, Wien *et al.* (2004) subjected onion to 50 % leaf herbivory and reported that the additional growth of leaves coupled with their increased photosynthetic rate suggested that leaf loss due to hail storms or diseases may have less impact on yield as long as the damage occurs early enough in the plants life so that compensatory growth can occur and the disease-causing organism is controlled.

1.5 Influences of drought stress on plant growth and development

Plant growth and productivity is generally known to be adversely affected by drought and other abiotic stresses. Generally, plants have some protective adaptations and certain strategies are put in place when they are subjected to drought stress. Some of these strategies may involve both protection and repair mechanisms (Bewley, 1995). As for instance, drought-stressed plants are known to survive dehydration resulting from drought by limiting aerial growth but increasing root development. Plants subjected to drought stress also accumulate compatible solutes to lower the water potential of their cells and enhance an inward movement of water into their cells (Scott, 2008b; Volaire,

2002). Blum and Ebercon (1981) also stated that maintenance of membrane stability is an important adaptive trait because cell membranes appear to be the main site of dehydration injury (Leopold *et al.*, 1981, McKersie and Leshem, 1994). According to Kuang *et al*. (1995) responses of plants to dehydration resulting from drought stress may involve the activation of a number of genes. Some genes encode for polypeptides with substantial homologies to proteins expressed during late embryogenesis (Bray 1993, Bartels *et al*. 1996). Dehydrins are a subfamily of 'late embryogenesis abundant' (LEA) proteins and they may accumulate in some dehydrated species (Close, 1996, Ismail *et al.*, 1999). According to Lvov and Fichtengolz (1936), during drought, mesophytic plants experience a rise in their respiratory rates. These plants maintain the water content of their protoplasmic colloids using water released by their respiration. Also, there is an accumulation of soluble carbohydrates in the leaves of non-irrigated plants in periods of drought and this is attributable to an inhibition of translocation. He explained that during soil drought the leaves of many mesophytes accumulate sugars and this is accompanied by decreases in the amount of phosphorylated compounds. In general drought stressed plants suffer from dehydration of its cells and tissues as well as from a considerable increase in the temperature of their body. This implies that, the absence of available water caused by drought is aggravated by high body temperatures of plants. The harmful effect of water stress therefore gives rise to reduced growth, especially during cell elongation, which also may decrease yields (Alexseev, 1950; Maximov, 1939). It follows that plants which have survived drought are also small and weak. Some plants may, however, react to progressive dehydration by changing the colloidal chemical state of their protoplasm, namely, by increased hydration and hydrophily of the colloids of the protoplasm (Badanova, 1960; Gusev, 1959; Henckel, 1946). Ratner (1944) concluded that water stress results in the same changes in the colloidal system as cell ageing because it lowers the water-holding capacity and the ability to swell.

Ilyin (1957) stated that during dehydration, injury and death of cells occur because there is mechanical disruption of protoplasm. Sisakyan (1940) revealed that the injury and death of plants from drought was due to excessive protein decomposition. Lepeschkin (1938) also confirmed that plants exposed to drought may experience a destruction of protein molecules. Paech (1934), however, disputed that, decreased protein is not a cause but a result of the injury and death, but Stocker (1958) disclosed that the main

cause of injury and death during water stress conditions was due to variations in the sub microscopic structure of protoplasm. In his opinion, drying of soils loosens specific molecular connections in the sub microscopic protoplasmic network. Bogen (1948) reported that it is a modification of the sub microscopic framework of the protoplasm and is accompanied by changes in viscosity, permeability, hydration, and electric charge as well as activation and deactivation of enzymes. Changes in metabolism at the reaction stage would result in alterations in the cells. Water stress may also cause an inhibition of the normal carbohydrate-phosphorus metabolism and this is consistent with the observation that drought first of all disturbs phosphorylation (Zholkevlch, 1960). The loss of water from plant tissues under drought conditions resulted in growth inhibition and in a number of other metabolic and physiological changes such as accumulation of abscissic acid (Zeevart and Creelman, 1988); stomatal closure and reduced transpiration rates, reduction in growth and productivity (Pelah *et al*., 1997). Hsiao (1973) also stated that plants subjected to drought stress may also have a decrease in the water potential of the plant tissues and reduced photosynthetic rates. There is also a synthesis of new proteins and mRNAs, and accumulation of osmolytes (Scott, 2008; Skriver and Mundy, 1990). Under drought conditions, survival of plants may depend on how long the tissues could maintain cell integrity at a given moisture content than on the actual minimum threshold of dehydration reached by the tissues.

1.6 Effects of planting depth and nutrient supply on growth of plants

Planting depth has proved to be an important agronomic factor that affects growth, development and yields of plants (Vogel, 1963; Stickler, 1962) but the optimum depth is dependent on plant type, size of planting material and the prevailing environmental conditions. In most cases, the influence of depth of planting on growth and development has been expressed in terms of germination or emergence, earliness, plant size and total yield. In commercial geophytes, most of the publications on depth of planting deal with depths ranging from 0 to 20 cm (Negbi *et al*., 1989). Galil (1961) studied the effects of different planting depths on a number of geophytes and reported that geophytic plants in their natural habitat have to struggle constantly to maintain their required depth, and by so doing a number of their characteristics are affected. He distinguished between linear depth that is the actual depth of the plants measured in millimetres or centimetres and absolute depth which is also referred to as the physiological depth. According to him,

the absolute depth comprises the array of physiological conditions to which the plant is exposed. He concluded that most bulbous plants and other geophytes move horizontally or/and vertically in the soil. Hagiladi *et al.* (1992) also stated that in agriculture as well as ornamental horticulture, growers may plant at certain depths but because these depths may not be the optimal planting depths for such crop plants, growth and survival of the plants may be negatively affected. They observed that an increase in planting depths of a number of geophytes delayed emergence and reduced the percentage of emerging plants. Also the number of developing shoots, leaves and flowering stems, number of daughter bulbs or corms, and their fresh and dry weights were negatively correlated with planting depth. Alam and Locascio (1965) studied the effects of planting depth and seed size on broccoli and beans. They found out that a reduction in yield occurred from deep planting of small seed compared with large or medium seed planted at the same depth. In a similar study, Kariuki (2003) observed that the average number of shoots, visible leaves and the length of inflorescence decreased with increasing planting depth, but planting depth had no significant effect on flower quality. Leopold (2007) also stated that too shallow planting of some geophytes will result in the plants responding to these shallow planting conditions by literally "pulling" themselves down into deeper ground.

The importance of plant mineral nutrition to its growth and development cannot be overemphasized. The three most important nutrients elements that are important (essential) in plant nutrition are nitrogen, phosphorus and potassium. The responses of hyacinth and the lily to nitrogen and phosphorus based nutrients were investigated as part of this study and results are discussed in chapter five of this thesis. Scott (2008) stated that nitrogen is so important to the growth and development of plants that its deficiency generally results in chlorosis that is the yellowish coloration of older leaves due to the fact that the nutrient is recycled from them to enable new leaves to grow. In plants, nitrogen is a constituent of other plant compounds such as chlorophyll and nucleotides. Enzymes are proteinaceous and this implies that nitrogen plays a key role in many metabolic processes in plants. The element is a structural constituent of plants' cell walls (Havlin *et al.*, 1999). Research on bulbous plants showed that deficiencies in nitrogen led to development of small plants and bulbs with early maturity. On the other hand, excess application of nitrogenous fertilizer has been known to produce softer bulbs which are more susceptible to rotting and delayed maturity (Sutcliffe and Baker,

1974; Tsutsui, 1975; Laughlin, 1989; Maier, Dahlenburg and Twigden, 1990; Bennett, 1993 and Clemens *et al*., 1998). The application of nitrogen-based fertilizers for bulb production is important especially on sandy soils where leaching of nutrients could be high. In such situations, multiple applications of smaller amounts of the element are most efficient in reducing nitrate losses through leaching. Batal *et al*., (1994) and Diaz-Perez *et al*. (2003) stated that a third of the total applied nitrogen should be applied early enough in the growing season (1-8 weeks after planting) and the remaining two thirds of the nitrogen applied late in the growing season (16-24 weeks after planting). This will allow nitrogen availability not only during the vegetative but also during the reproductive phase of most bulbous plants. They stated that the excessive use of the nutrient influences the balance of other nutrients in the soil solution. This normally result in nutritional disturbances that may give rise to acute symptoms, but more often remain latent and materialise only as subnormal growth and quality (Bergmann, 1992). Nitrogen is absorbed by plants either as NH_4^+ or NO_3^-. The former is absorbed and utilised primary by young plants, whereas the latter is the main form utilised during the late growth stages, however, plants vary in their proportion of NH_4^+ and NO_3^- utilisation (Bennett, 1993). After plants have absorbed either the ammonium or nitrate from the soil, these ions are transformed in the plants to the amine form. It is then utilised to form amino acids which are essential for protein formation because they are their building blocks. Amino acids are also part of the nucleic acids, DNA and RNA that are responsible for the genetic information in plants and also direct protein synthesis (Bergmann, 1992).

Phosphorus is also obtained in the soil as a soluble phosphate or as hydrogen phosphate salt. The element may be absorbed by plants as $H_2PO_4^-$ or as HPO_4^{2-} depending on the pH of the soil. Phosphorus plays an important role in plants as it is also a constituent of plant enzymes and proteins and a structural component of phosphoproteins, phospholipids and nucleic acids. As a component of nucleic acids and genes, phosphorus plays a role in reproduction. It enables fruit to mature early and also improves their quality (Scott, 2008a; Bennett, 1993). Phosphorus is a part of NADP and helps in the photosynthetic process. It helps in energy storage and transfer through the compounds ADP and ATP. It is also involved in electron transport in oxidation-reduction reactions. Phosphorus plays a regulatory role in the formation and translocation of substances such as sugars and starches. It is also important in the

maturation processes, seed formation and symbiotic nitrogen fixation. Deficiency of phosphorus in the soil gives rise to dark green coloration of leaves of plants, stunted growth, necrotic spots and accumulation of anthocyanin pigments in leaves of plants (Scott, 2008a).

Roodbol *et al*. (2002) studied the effects of KNO_3, Ca $(NO3)_2$, $MgSO_4$ and $(NH4)_2SO_4$ on bulb yield and quality of *Lachenalia*, a species in the family *Hyacinthaceae*. They reported that nutrition in the previous season as well as the season when the plant flowers both influenced the number of florets per inflorescence. They also observed that the nutritional requirements of the bulb depended on cultivar type and that large bulbs require higher nutrient levels than small bulbs. In like manner, Silberbush *et al*. (2003) investigated into N and K fertilization interaction with CO_2 enrichment on the development of *Hippeastrum* bulbs grown in the greenhouse. They observed that the response in growth to both nutrients' concentration was curvilinear and that each of the nutrients contributed significantly to bulb growth but the optimal response of the larger bulbs was at a higher CO_2 concentration, for each of the two nutrients. Mahgoub *et al*. (2006) also reported that in Irish bulb, plant height, leaf fresh weight as well as dry weight, and inflorescence length increased when plants were fertilised with nitrogen at the rate of 40 g plus 30 g K/m^2. Number of bulblets also increased at nitrogen fertilizer application level up to 60 g/m^2 plus 25 g of K/m^2. Applying the nutrients at the rate of 30 g N plus 40 g K/m^2 or vice versa, improved the spike and inflorescence length. In onion, Ali *et al*. (2008) also studied the response of the bulb to cowdung and phosphorus application. They reported that the emergence, plant height, number of flowers per umbel, days to blooming, fruit set per umbel and seed yield were significantly influenced by these treatments but the number of tillers per plant was not affected by the application of any of the components or the combined application of the two components. The maximum seed yield and quality of the onion bulb was obtained when phosphorus was applied at 80-120 kg/ha plus cowdung. Also in *Freesia hybrida* bulb, Hamit (2001) reported that the application of either nitrogen or phosphorus increased the number of spikes per plant whilst the number of florets per plant was not affected by these nutrients. In lilies, Beattie and White (1993) observed that the bulb requires fertilizers that are high in potassium, nitrogen and calcium but low in phosphorus and magnesium whilst Treder (2000) also stated that different varieties of the bulb require different nutrient requirements.

1.7 Influence of bulb size at planting and varietal effects

In general, the performance of vegetatively propagated plants is influenced by the amount of stored reserves present in the tuber, corm, rhizome, or bulb at the time of planting. Rees (1969) investigated into the influence of bulb size at planting on growth and yield of tulip bulbs and reported that shoot weight, leaf area and weight of daughter bulb were directly related to the size of the mother bulb but are controlled by factors determining mother bulb size during its development. Also, the relative growth rates of smaller plants were higher than those of the larger ones but that of the daughter bulbs were unaffected by the size of mother bulb. Similarly, the larger bulbs lost weight more rapidly than the smaller ones. He stated that the above factors were influenced by locations and seasons. In potatoes, Burton (1966) reported that large planting material produced a higher total yield of tubers. Afonja (1967) also found higher yields of tubers from higher sets weights of white yam *(Dioscorea rotunda)* and Enyi (1967) obtained higher yields of cocoyam from plants grown from larger sets. Even on plants that are propagated by seeds, Black (1959) observed that the dry matter accumulation during the vegetative stage was positively related to seed weight. According to Watad *et al*. (1999), flowering in *Aconitium*, a member of family *Ranunculaceae*, was highly dependent on tuber weight. In this species, tuber size of 30-40 g produced flowers of good quality whereas plants produced from tubers of 5 g or less did not flower. Also, plants from larger tubers produced more tubers, whereas smaller ones recorded a higher growth rate.

Rees (1985) stated that bulbs below a certain critical size would fail to flower even when planted under favourable conditions. Above this critical size, flower quality that is number of flowering shoots, number of florets per inflorescence, and stem length often improve as size of the mother bulb increases. De Munk and Schipper (1993) also stated that Dutch Iris bulb must be of a certain critical size before they can produce flower. Fernandez *et al.* (2009), reported that quality and vegetative parameters were improved as bulb size at planting increased but these parameters were not affected by temperature treatments. Padhye and Cameron (2007) also observed that the influence of bulb size on time of flowering was variable and cultivar dependant. Ghaffoor *et al.* (2003) compared three onion varieties namely ‘Faisalabad Early’, ‘Phulkara’ and ‘Shah Alam’ in terms of their response to NPK fertilizer. At 150:100:50 NPK kg ha^{-1}, ‘Shah Alam’ gave the best performance in terms of number of leaves per plant, bulb survival, bulb diameter, marketable yield, culls percentage and total yield while ‘Phulkara’ produced the highest

plant height and leaf length. Jilani and Ghaffoor (2003) also screened ten varieties of onion for vegetative parameters and their yielding ability and observed that at seedling stage, number of leaves per seedling was the same in all varieties. Variety Shah Alam had the maximum number of leaves, leaf width, and leaf length but there was no significant difference in bulb diameter at harvest. Stahlschmidt *et al*. (1994) also researched into growth and yield of the following three cultivated varieties of garlic (*Allium sativum L.*): Blanco, Colorado and Rosado. They reported that there was no significant difference among the relative growth rates of the varieties during the initial phase where growth was slow. However, higher net assimilation rate of Rosado was counterbalanced by a lower leaf area ratio. In the second phase of logarithmic growth, plant relative growth rate was higher in Blanco and Colorado than in Rosado because the leaf area ratio of the former was high. During the last phase of linear growth, there was no difference in relative growth rates and the rate decreased in all the varieties. It was observed that the lower value of bulb dry weight in Rosado was as a result of the lower leaf area ratio that resulted from the cultivar's smaller photosynthetic apparatus.

1.8 Molecular biology and plant growth and development

The use of molecular biology or biotechnology to improve plant growth and development often involves the characterisation of cDNA clones encoding enzymes that influence certain desirable traits in the plant (Matthews *et al*., 1997). In general, partitioning and metabolism of carbohydrates has a great influence on the source-sink relationship as well as the entire growth and development of plants. In plants, studies at the molecular level on genes that govern or influence carbohydrate metabolism have been carried out using plant species such as *Ipomoea batata*, *Manihot esculenta*, *Arabidopsis thaliana* and *Hordeum vulgare*; and information on isolation, sequencing and expression of carbohydrate metabolism genes may provide understanding not only into the mechanisms involved in starch degradation and biosynthesis, but also the general biochemical principles involved in the regulation and partitioning of carbohydrates in plants (Kim *et al*., 2005; Miller, 1992). According to Kim *et al*. (2005), the development of the storage roots of geophytes normally coincides with starch accumulation in the organs, and genes that influence the synthesis and metabolisms of starch play an important role in the development of the storage roots as well as the storage sink strength. According to the research conducted by the above

authors, the gene for isoamylase-debranching enzyme is strongly expressed in developing tuberous roots of sweet potato and the activity of this gene positively correlates with the sink strength during the development of the root. Tangphatsornruang *et al*. (2005) also isolated an α-amylase gene, *MEamy2* from the tuberous roots of cassava, and reported that this gene occurs as a single copy in the cassava genome, and shares the highest homology with the amylase gene, AMY8 from apple fruit. According to these authors, the RT-PCR analysis showed that the gene expression of *MEamy2* was induced within two hours following the application of exogenous gibberellins but the gene expression was not influenced by treatment with abscisic acid. St Pierre *et al*. (1996) also reported that the transcription of starch phosphorylase gene in potato occurs essentially in the starch containing cells associated with the vascular tissues implying that starch phosphorylase plays a role in the mobilisation of starch stored along the translocation pathway. Work done by Salehuzzaman *et al*. (1993) revealed that the sequence generated from the starch synthase clone constructed from cDNA library of cassava from the tuber had 74% identity with potato starch synthase; however, the percentage identity of starch synthase from other plant species was 60-72.

1.9 Research objectives

The common hyacinth and the lily are important ornamental geophytes cultivated mainly for indoor and outdoor decorations. In recent years, there has been an increased interest and activity in geophytes especially in the area of carbohydrate metabolism because tubers, corms, roots and bulbs from this group of plants have the ability to store and remobilise reserved metabolites, particularly carbohydrates. Metabolism of carbohydrates in these species is essential to their growth, development and yield. In the common hyacinth and lily, however, information concerning biochemistry of carbohydrates metabolism especially on the regulation of carbohydrate partitioning and metabolism and the general aspects of growth and physiology is very limited. The present study was therefore undertaken to determine the changes in the concentration of the various carbohydrates particularly starch during the growth of hyacinth and the lily, either in relation to their utilization in the mother bulb scales, or their accumulation in the newly formed organs. The study also sought to investigate into the relationship between starch and sugars on one hand, and the distribution of activities of enzymes that

are involved in starch metabolism within the bulb of these plants at various stages of growth, on the other hand. Also, among the factors that influence carbohydrate partitioning and metabolism in plants is the gene expression of the enzymes that are involved in the biosynthesis and degradation of the carbohydrate. Thus at the molecular level, studies were initiated with a view to generating sequence information from some of the enzymes that are involved in starch metabolism in the common hyacinth. This information will be useful because it will help to investigate the expression of these genes by using specific probes for RT-PCR. In plant production, the relationships between assimilate supply and demand is best studied when the sources of assimilates are removed. The underground portion of flowering bulb as well as the photosynthetic leaves could behave as sources of assimilates depending on the stage of growth and development of the plant. In this study, both the leaves and bulbs of hyacinth and lily were subjected to simulated herbivory, with a view to studying the effects of these treatments on growth, inflorescence development and yield of the plants. Additionally, water stress is known to be detrimental to plant growth and development. Plants from the two bulbous species were subjected to drought during part of their growth in the plant house, with a view to understanding the physiological mechanisms of these species to cope with this abiotic stress. Moreso, in order to produce good quality plants for consumers, growers normally ensure that certain agronomic practices are put in place during the cultivation of crop plants. The study, therefore, also aimed at investigating into the influence of some agronomic practices such as nutrients application, depth of planting, bulb size at planting and varietal effects on the productivity of these plants.

CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals

The chemicals used in this study were magnesium chloride, Hepes, soluble starch, Tris-HCl, glycerol, ß-mercaptoethanol, Bovine serum albumin (BSA), Hepes-HCl, EDTA, ADP glucose, amylopectin, Phosphoenolpyruvic acid (PEP), KCl and insoluble PVP. The other chemicals used were Ammonium sulphate, Glucose-6-phosphate, iodine reagents, 2-[N-morpholino] ethanesulphonic acid (MES), Calcium chlorides, HCl and limit dextrin. These chemicals were also purchased from Boehringer, Roche Diagnostics, UK. In addition to the above chemicals, some enzymes were purchased from Sigma, UK, for use in this study. These enzymes were amyloglucosidase, α-amylase, ß-fructosidase (invertase), hexokinase, glucose-6-phosphate dehydrogenase, phosphogluco isomerase, phosphoglucomutase, lactate dehydrogenase and pyruvate kinase. Similarly ATP, NAD, NADH and NADP were used in the study and they were purchased from Sigma, UK.

2.2 Plant materials

The planting materials used in the study were bulbs of the common hyacinth, *Hyacinthus orientalis* and those of the lily, *Lilium longiflorum*. The hyacinth varieties used were Purple Voice, Jon Bos, Sky Jackets, Pink Pearl, Fondant, Blue Jacket, Amethyst and Splendid Cornelia whilst in the case of the lily, the Longiflorum hybrids were used. They were purchased from the Spalding Plant and Bulb Company, UK.

2.3.0 Design and set up of experiments

Eight areas of study were carried out in the Plant Stress Unit of the Biology and Environmental Sciences Department, School of Life Sciences, Sussex University, between October 2006 and June 2010, using bulbs of the common hyacinth and lily as test plants. These areas of study were as follows: regulation of carbohydrate partitioning and metabolism, responses of the plants to simulated leaf and bulb herbivory, influence of drought stress on growth and development of the plants, isolation and sequence analysis of the hyacinth starch metabolism genes, effects of various planting depths on growth and productivity, influence of bulb size at planting, bulbs and nutrient supply, and varietal effects on growth and yield. Results on carbohydrate metabolism were

stated and discussed under Chapter three of this thesis, whilst the response of the plants to herbivory and drought stress were also discussed under Chapter four. Chapter six was on molecular studies (i.e. Isolation and sequence analysis of hyacinth starch metabolism genes), and the last four areas of study were captioned 'Some agronomic practices' and discussed under Chapter five. In general, the experimental design used was the same for all the experiments carried out in the greenhouse: bulbs were planted in plastic pots of capacity 0.01 m^3. Prior to planting of these bulbs, pots were filled with compost and perlite in a ratio of 2:1 by volume and watered; and after planting, the units were arranged on greenhouse benches, and treatments replicated using randomised complete block design (RCBD).

2.3.1 Carbohydrate partitioning and metabolism studies

On 9th November 2006 that is during the 2006/2007 planting season, hyacinth bulbs of average fresh weight 60 g were planted. Before planting and at 1, 2, 3, 4, 5, 6 and 7 months after planting, all bulbs in a pot were carefully uprooted and used for carbohydrate analysis. During measurements, bulbs were peeled into seven scales as L1, L2, L3, L4, L5, L6 and L7 where L1 refers to the outermost scale and in that order to the very innermost scale (L7). Each of these components was used for starch, glucose, fructose and sucrose measurements using the method described by Morrell and Rees (1986). Contents of the last pot were harvested for analysis on 12th June 2007. Total fresh weight of each scale was determined at each stage of growth using electronic weighing balance. Dry weights were determined by oven drying the samples at 80^{o}C for 24 hours and moisture content calculated as the difference between the two weights expressed as a percentage of the fresh weight. At the end of this experiment, scales L1–L4 were grouped together as outer scales because they exhibited similar metabolic functions (See chapter 3). Similarly, scales L5-L7 were also grouped together as inner scales for further studies. Thus on 29th October 2007, that is during the 2007/2008 planting season, 40 hyacinth bulbs of similar fresh weight were planted, and before planting and at 2, 4, 6, 8, 10, 12, 14 and 16 weeks after planting, the bulbs were uprooted and dissected into outer scales (L1–L4), inner scales (L5 - L7 scales), newly formed leaves, stem (basal plate) plus roots and flower for starch measurement. Fresh weights, dry weights and moisture content were determined as in 2006/2007 season.

Figures 2.1 and 2.2 show the appearances of the hyacinth bulbs at the various stages of growth during the experimental period. Since results from the 2006/2007 and 2007/2008 seasons revealed that the scales, particularly the outermost ones lost a lot of starch and biomass during the first month after planting, in 2008/2009, another experiment was set up to investigate into this loss in biomass and starch that characterised the early stages of development of the bulb after planting. Therefore on 14th October 2008, bulbs of similar fresh weights as the previous studies were planted using the same composition of the compost and perlite (herein referred to as soil). During the same period, another set of bulbs were planted in glass tubes. In using the glass tubes, the tubes were filled with water and the bulbs placed on top of the water. At 0, 4, 8, 12, 16 and 20 days after planting in soil and in glass tubes, the bulbs were taken from each category for starch measurements of the various bulb parts: outer scales, inner scales, new leaves, stem (basal plate) plus roots and flower.

The carbohydrate studies on the lily bulbs started on 16th April, 2008 (planting) and ended on 13th August 2008 (harvesting). In this study, bulbs of average fresh weight 30 g were planted in the same manner as those of hyacinth. At 0, 3, 6, 9 and 12 weeks after planting, the bulbs were uprooted and dissected into outer scales (L1–L10), inner scales (L11- L20), newly formed leaves, stem (basal plate) plus roots, and flower for both sugar and starch measurements as in the hyacinth experiments. Fresh and dry weights of the scales were determined as in the case of hyacinth. Details of these studies are presented and discussed under chapter three.

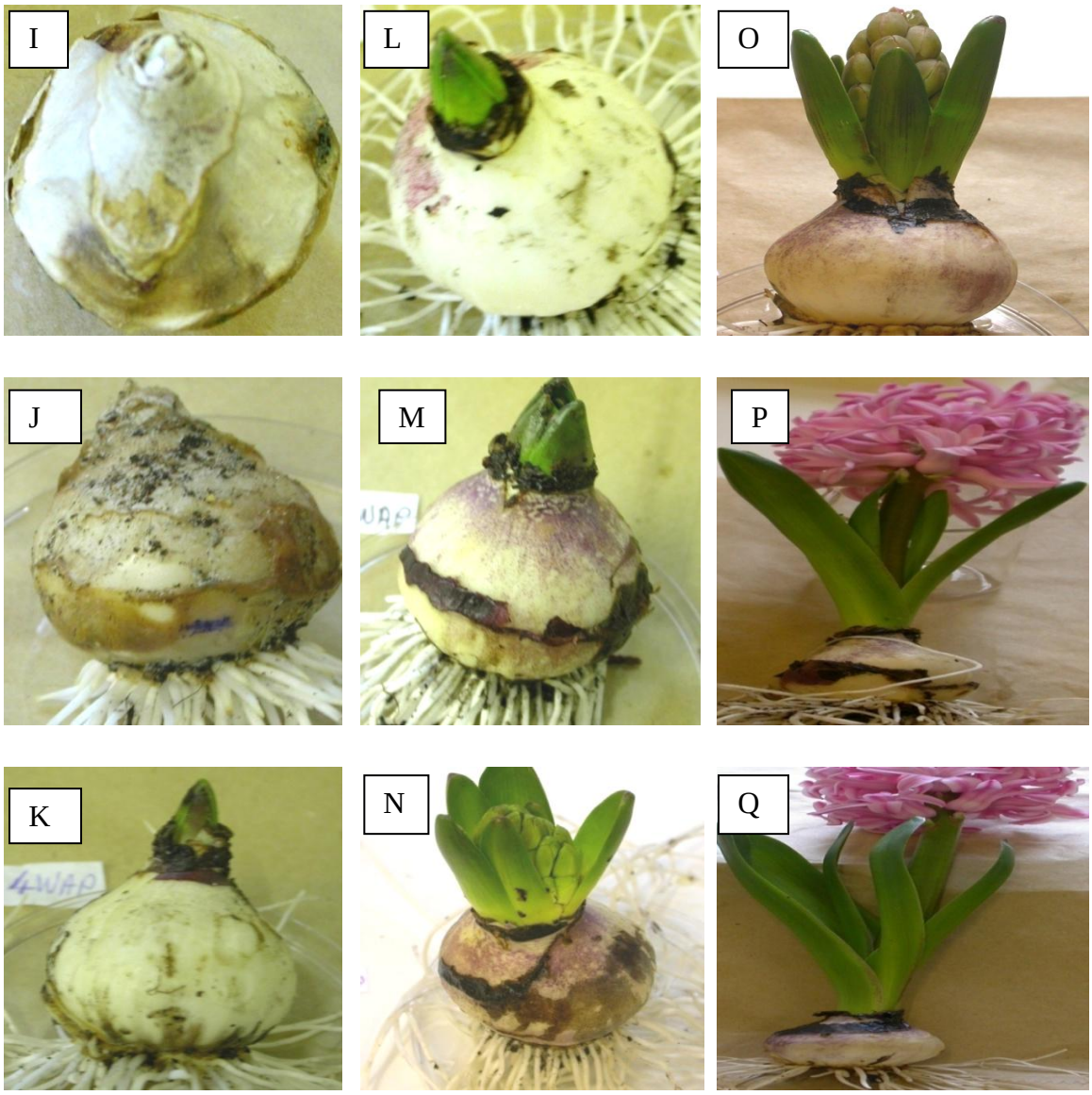


Fig 2.1: Growth and developmental stages of the common hyacinth. Letter 'I' is the appearance of the bulb before planting whilst J, K, L, M, N, O, P and Q are the developmental phases at 2, 4, 6, 8, 10, 12, 14 and 16 weeks after planting respectively.

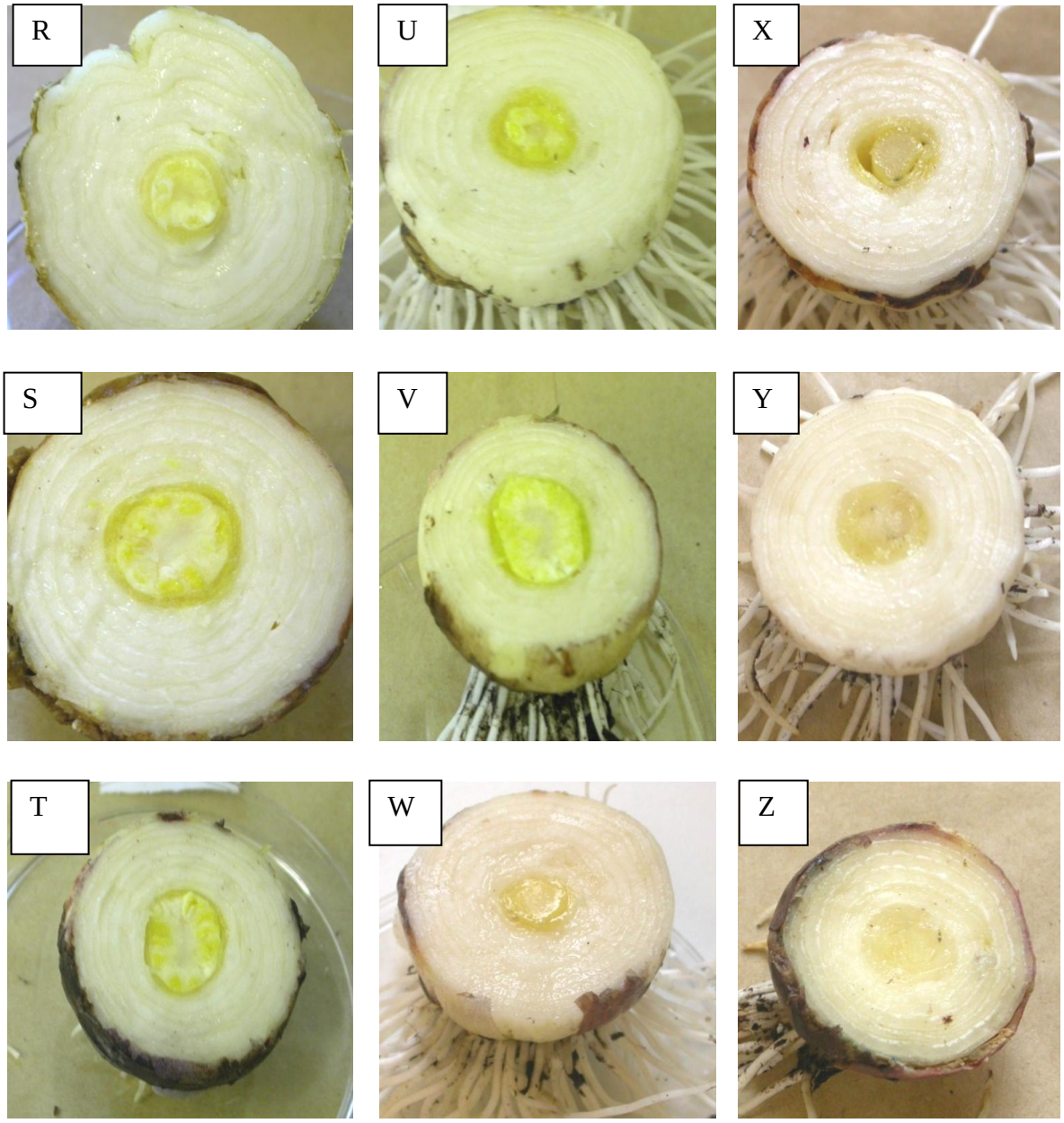


Fig 2.2: Photographs of transverse sections of bulbs of the common hyacinth at the various stages of growth and development. Letter 'R' is the cross section of the bulb before planting whilst S, T, U, V. W, X, Y and Z are the sections made at 2, 4, 6, 8, 10, 12, 14 and 16 weeks after planting respectively.

2.3.1.1 Measurements of carbohydrates and enzymes activity

In measuring carbohydrates and enzymes activity, assays generally made use of methods involving reactions that basically converted NAD to NADH. Measurements were made in an ELISA plate reader (Anthos HTLL spectrophotometer that uses Delta Software). Calculations were based on the extinction coefficient of NADH/NAD at 340

nm. In general, a base rate was established prior to starting the reaction. The reaction was left for about 30 min before another set of readings was made. The difference between the readings was directly related to the amount of enzyme used in the conversion of NAD/NADH (Seals, 2003).

2.3.1.2 Determination of starch content

The procedure of this assay was to digest the samples into glucose units and assay for the glucose. Fresh samples of the various plant parts after dissection (L1, L2, L3, L4, L5, L6 and L7 of hyacinth scales; outer scales, inner scales, newly formed leaves, stems (basal plate) plus roots, and flowers of both hyacinth and the lily) were immediately frozen in liquid nitrogen. They were blended thoroughly and 10 ml of distilled water added. The mixture was autoclaved for two hours to solubilise the solution and digested at 37^{o}C for four hours using 100 µl of 100 mM sodium citrate at pH 4.8, added to 15 µl and 0.6 µl of amyloglucosidase and α-amylase, respectively. Then to each 50µl of the starch sample from above, 125 µl of 100 mM Hepes, 25 µl of 40 mM $MgCl_2.6H_2O$, 25 µl of 50 mM ATP, and 25 µl of 2 mM NAD were added. One unit of hexokinase was added prior to recording the base absorbance readings at 340 nm on a spectrophotometer. Then one unit of glucose-6-phosphate dehydrogenase was also added before the second reading was made. Values obtained were entered into a spreadsheet, and the formula according to Morrell and Rees (1986) was used for the calculation of the starch content.

2.3.1.3 Determination of soluble sugar content

The samples of the various scales and organs as detailed above were boiled in 100 % as well as 50 % ethanol, and then in distilled water for 4 minutes, separately and extracts from these treatments poured into a flask and labelled as first extraction. The procedure was repeated as second and third extractions. The content of each flask was evaporated to dryness using the rota vapour machine. An amount of 1 ml distilled water was pippetted into a flask containing dry samples and shaken with some glass beads for 4-5 minutes. The sample was digested at 37^{o}C for four hours using 100 µl of 100 mM sodium citrate at pH 5.6, 8 units of invertase (β-fructosidase) and 100 µl of the sugar

sample. Then to each 50 µl of the sugar sample from above, 125 µl of 100 mM Hepes, 25 µl of 40 mM $MgCl_2.6H_2O$, 25 µl of 50 mM ATP, and 25 µl of 2 mM NAD were added. Then, one unit of hexokinase was added prior to recording the base absorbance readings at 340 nm on a spectrophotometer, followed by another one unit of glucose-6-phosphate dehydrogenase before taking the second reading. One unit of phosphoglucose isomerase (PGI) was also added for the third reading to be made. Values obtained were entered into a spreadsheet for the calculation of all the various reducing sugars.

2.3.1.4 Measurements of enzymes activity

Bulbs of the common hyacinth were used in these measurements. Studies started on 30th September, 2009 when the bulbs were planted, and ended on March 2010 where the last measurement was made. At 1, 2, 3, 4, 5 and 6 months after planting, the plants were carefully uprooted and dissected into the various organs: outer scales, inner scales, newly formed leaves, stem (basal plate) plus roots, and flowers. These samples were then frozen before measurements were made.

(a) α-amylase

The method used in these measurements was adapted from Adams *et al.* (1981). Buffer to tissues ratio of 1: 3 was used. The samples were blended and extracted using an extraction buffer that comprised 0.05 M MES at pH 5, 0.02 M $MgCl_2$, and 0.02 M $CaCl_2$. The mixture was centrifuged at 30,000 g for 10 min and the supernatant was kept on ice the same day for the assay. The reaction mixture contained 0.2 % soluble starch, 0.2 % limit dextrin, 0.5 M Citrate buffer at pH 6 and the enzyme extract. The mixture was incubated at 25^{o}C for 20 min and dilute HCl was added to stop the reaction. An iodine reagent was added before measurement was made on a spectrophotometer at a wavelength of 340 nm.

(b) Starch phosphorylase

The method used was modified from that used by Oluoha and Ugochukwu (1991) and Locy (1998). Plant samples (10 g) were homogenised in 15 ml of the extraction buffer containing chilled 0.5 M sodium citrate buffer pH 6.5, 0.4 M insoluble PVP and 1mM EDTA and the homogenate centrifuged at full speed for 10 mins. The supernatant was

30 % saturated with $(NH_4)_2SO_4$ and allowed to dissolve by stirring continuously for about 30 mins whilst the mixture was kept cold by putting it on ice. The mixture was again centrifuged as before, the supernatant was discarded and 500 µl of distilled water was added and shaken gently to dissolve the precipitate. This mixture was kept on ice for measurements the same day. An amount of 50 µl of 0.2 % soluble starch was added to 50 µl of 10 mM glucose-phosphate in 0.1 M Citrate buffer at pH 6.5 and incubated with 100 µl of the enzyme extract at 28^oC for 10 min. Then 50 µl of an iodine reagent was added before measurement was made.

(c) ADP glucose pyrophosphorylase

The method employed in this measurement was adapted from Rochat *et al*. (1995), Dorion *et al*. (1996), Pelleschi *et al*. (1997) and Seal (2003). The plant tissue was blended and the homogenate suspended in 100 mM Tris- HCl pH 8 containing 10 % glycerol, 0.1 % ß-mercaptoethanol and 0.1 % bovine serum albumin (BSA). The mixture was centrifuged at 4^oC at 10,000 g for 10 min and the supernatant kept on ice for assay the same day. ADP glucose was assayed in the direction of ADP glucose break down. During measurement, 50 µl of the enzyme extract from above was added to 90 mM Hepes-HCl at pH 7.4, 3 mM $MgCl_2$, 0.6 mM EDTA, 0.8 mM ADP-glucose, 1.1 mM NAD, 0.6 units phosphoglucomutase and 0.5 units glucose-6 phosphate dehydrogenase. The absorbance at 340 nm was read as a base reading and 0.7 mM PPi was added to start the reaction for about 30 mins before another reading was made.

(d) Soluble starch synthase

In this method, ADP glucose was used to synthesize amylopectin and ADP, and assaying for the ADP produced. It was adapted from Nakamura *et al*. (1989) and Scott (1992). The same extraction buffer and procedures were used as in the case of ADP glucose pyrophosphorylase. Thus 50 µl of the extract kept on ice from (c) above was added to 50 mM Hepes-HCl pH 7.4, 0.896 mM ADP glucose, 0.665 mg amylopectin in a total volume of 250 µl and incubated in two samples at 25 and 0^oC for 20 min. The samples were then centrifuged at 12, 000 g for 2 min. Then, 50 µl from the incubated samples above was added to100 mM Hepes at pH 7.6, 1 mM PEP, 40 mM $MgCl_2$, 0.2 mM KCl, 0.36 mM NAD, 1.5 units lactate dehydrogenase in a total volume of 250 µl. After 10 min, measurement was made at 340 nm and 0.4 unit pyruvate kinase was

added. The reaction was left at room temperature for one hour before another set of readings was made.

2.3.2 Simulated herbivory and drought stress

In this study, both the above-ground (leaves of hyacinth, or the complete shoot system in the case of the lily) and below-ground (the bulb) were subjected to herbivory treatments to study the response of the plants to these stresses. Also, plants produced from either peeled or unpeeled bulb were drought stressed and their performances compared with the unpeeled control.

2.3.2.1 Below-ground herbivory

Two sets of experiments were conducted on the common hyacinth on simulated bulb herbivory. The first experiment was planted on 9th November 2006 and harvested on 12th June 2007. Plant arrangements on greenhouse benches and design remained the same as those stated under section 2.3.0. Prior to planting and at 2 and 3 months after planting, half (50 %) of all the scales tissues of each bulb were removed by peeling and the plants produced from the unpeeled bulbs were used as control. In the second experiment, the hyacinth bulbs were planted on 14th October 2008 and harvested on 4th June 2009. Before planting and at two weeks after planting, either half of all the scale tissues of each bulb or all the scales of the whole bulb were removed by peeling (100 % scale removal or complete bulb scale removal). Figure 2.4 shows the appearance of the plants produced from peeled bulbs (i.e. a reduction in growth rate of hyacinth as a result of partial scale removal as compared to those of the unpeeled control). The lily experiment on the response of the species to below-ground herbivory was set up in the same manner as that of hyacinth during the 2008/2009 season. Bulbs were planted on 16th April 2008 and harvested on 13th August 2008. That is, the lily bulbs were also subjected to both 50 and 100 % scale removal treatments prior to planting and at two weeks after planting.

2.3.2.2 Above-ground herbivory

The leaf herbivory studies in hyacinth were conducted at the same time as those of bulb herbivory that is between October 2008 and May 2009. Plants were planted as mentioned under section 2.3.0, and at 14 weeks after planting either all leaves (1st 100 % defoliation) or half of each leaf on each plant (1st 50 % defoliation) were manually removed. Similar leaf removal activities were carried out at 18 weeks after planting (2nd 100 and 50 % defoliation). Figure 2.3 shows the appearance of the plants just after leaf herbivory. The above-ground herbivory studies in the case of the lily were also carried out during the summer period of April to July 2008. In the case of the lily experiment, shoot removal occurred at three and five weeks after planting for first and second shoot removal treatments, respectively. During shoot removal, either the whole shoot or half the shoot system of each plant was artificially removed (100 and 50 % shoot removal, respectively). Figure 2.5 shows the appearance of the lily before and after complete shoot removal.

2.3.2.3 Drought Stress

Drought stress studies on hyacinth were carried out between 7th November 2007 (planting) and 19th May 2008 (harvesting). In this experiment, plants produced from 50 % peeled bulbs and those from unpeeled bulbs were either watered throughout the season (control) or drought stressed from 4-24 weeks after planting. Studies on response of the lily to drought stress also started from April to August 2008, and it was set up similarly to that of hyacinth, but in this case, water stress started at 3-12 weeks after planting.

2.3.3. Effects of some agronomic practices on growth and productivity of the bulbs

The responses of hyacinth and lily to the following agronomic practices were investigated: depth of planting, nutrients application, bulb size at planting and varietal effects. Detailed results of these studies are shown in chapter five. Plant arrangements and design used in these experiments were the same as those stated under section 2.3.0.

2.3.3.1 Depth of planting

Investigations into the influence of planting depth on growth and yield of hyacinth were conducted from October 2008 to June 2009 whilst that of the lily took place between April and November 2009. The initial fresh weights of bulbs prior to planting were 25-30 g and 66-70 g, respectively for hyacinth and the lily. In all cases, bulbs were planted at 0, 5, 10, 15 and 20 cm depths from soil surface.

2.3.3.2 Nutrients application

Studies on response of the bulbs to nutrients application occurred from October 2008 to June 2009 for hyacinth, and April 2009 to November 2009 for the lily. The following nutrients: $(NH4)_2SO_4$, Na_2HPO_4 and Na_2SO_4 were applied on weekly basis, each at 30 mM, 60 mM or 90 mM starting from 10 weeks after planting in the case of hyacinth, and 3 weeks after planting in the case of the lily. Plants that received no nutrients application were considered as control.

2.3.3.3 Bulb size at planting

Investigations into the influence of bulb size at planting on growth and productivity of the species were conducted at the same time as studies involving planting depth and nutrients application. In the case of hyacinth, bulbs of the following fresh weight: 10, 20, 30, 40, 50, 60, 70 and 80 g were planted, whilst for the lily, bulbs were grouped into the following sizes for planting: 10-19, 20-29, 30-39 and 40-49 g.

2.3.3.4 Influence of varieties on growth and yield

Eight varieties of the common hyacinth were planted on 30th September, 2010 for evaluation in terms of their ability to produce good quality flower, bulb yield or bulblets production. The varieties considered were Purple Voice, Jon Bos, Sky Jackets, Pink Pearl, Fondant, Blue Jacket, Amethyst and Splendid Cornelia.

(a)

(b)

Fig 2.3: Plants of the common hyacinth subjected to (a) complete and (b) partial defoliation at 14 weeks after planting during the 2008/2009 season.

Fig 2.4: Variation in plant sizes as a result of 50 % scale removal of hyacinth bulb prior to planting. On the left, Control and right, plants produced from partially peeled bulbs of hyacinth prior to planting during the 2006/2007 season. Photographs were taken when plants were 12 weeks old.

(a)

(b)

Fig 2.5: Response of the lily to complete (100 %) shoots removal: (a) complete shoot removal at 3 weeks after planting and (b) the appearance of the plants in (a) at 9 weeks later (Lily compensated for leaf loss by producing new but longer leaves. Chapter 5 discusses this in details).

2.4 Methods of data collection

A number of pertinent measurements were made during data collection. The methods used in collecting these data and the types of data collected for analysis are presented below:

2.4.1 Days of emergence, leaf length, leaf width and leaf area

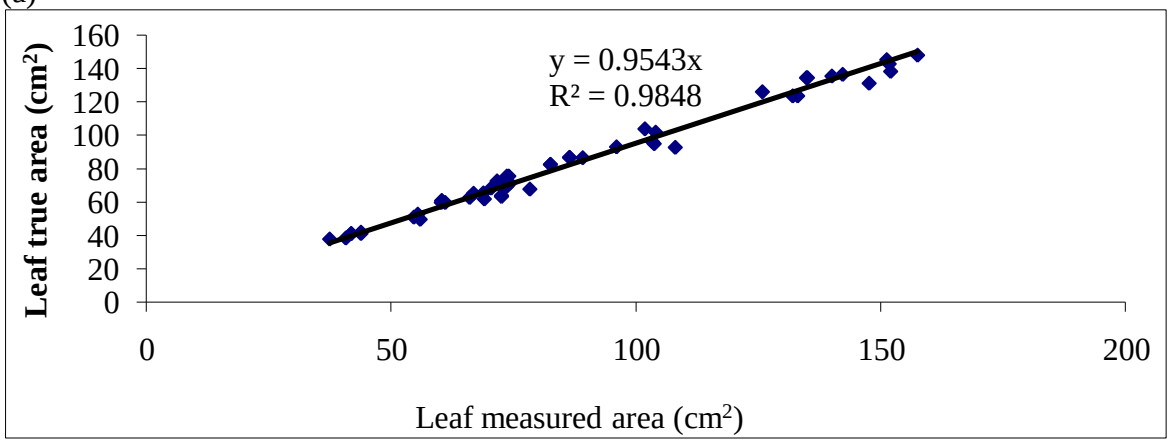
Days of emergence of bulbs from the soil following planting was recorded in the experiment on simulated bulb herbivory. It was measured as the number of days taken for either the peeled or unpeeled bulbs to emerge after planting. Leaf length was measured as the whole length of the leaf from the base to the leaf tip, whilst leaf width was measured as the distance at the middle section of the leaf where it is broadest. Total leaf length and width were recorded as the sum of all the individual leaf lengths and widths for one particular plant. Total leaf area was deduced from leaf length and width as described under section 2.4.1.1.

2.4.1.1 Determination of leaf area

Forty leaves of hyacinth, and also from the lily from plants already growing in the greenhouse were obtained through a destructive sampling. The product of the length and broadest part of the leaf (width) was recorded as measured leaf area (MLA) for all these leaves. Then, outlines or shapes of these leaves were sketched on A-4 papers and cut out with a pair of scissors. These pieces of papers made from the A-4 papers were weighed separately using an electronic balance and the weight of each piece of paper recorded as leaf paper weight (LPW). The lengths and widths of three of such A-4 papers were multiplied to get areas of these A-4 papers. The average area of the three A-4 papers was divided by their average weight to get a constant that was multiplied by the weight of each piece of paper (LPW) corresponding to the leaf outline to get the leaf true area (LTA). Finally, a graph of LTA was plotted against MLA. A line of best fit was made to pass through the points and the gradient of this line served as leaf area constant for each test plant as shown in Figure 2.6. The leaf area constant for hyacinth was 0.9543 (Fig 2.6a) whilst that of lily was 0.8921 (Fig 2.6b). Thus for hyacinth, leaf area (LA) for one particular leaf was calculated as MLA x 0.9543 and the total leaf area for the whole

plant is the sum of (MLA x 0.9543) of all leaves in the plant. In the case of the lily, four leaves from different positions of each plant: lower part of the shoot, middle section, towards the top of the plant and at the shoot tip were considered in the computation of MLA x 0.8921 and average value obtained. Thus the total leaf area for the plant was calculated as Average of (MLA x 0.8921) x total number of leaves on the plant at that time.

(a)



(b)

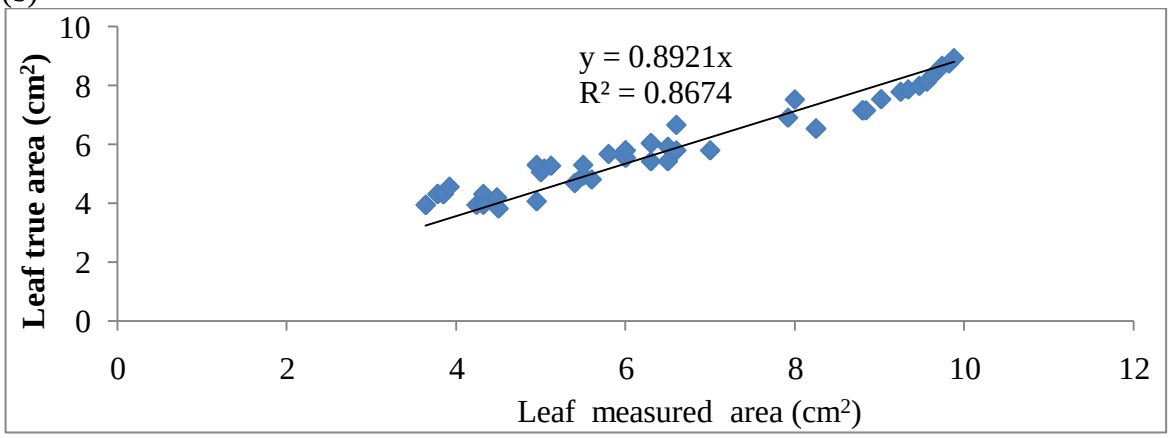


Fig 2.6: Determination of leaf area constant for (a) the common hyacinth and (b) the lily.

2.4.2 Inflorescence development

Inflorescence development was measured in terms of inflorescence length, inflorescence diameter, inflorescence stalk diameter, peduncle length and number of florets. In hyacinth, inflorescence height was recorded as the distance from the soil surface level of each plant to the tip of the inflorescence. In the lily, it was measured as the distance from soil surface to the tip of the tallest flower. Inflorescence length was taken as the inflorescence height minus the length of the stalk holding the inflorescence. Peduncle length or the length of the flower stalk was measured in the lily as the length from the point of attachment of the flower from the shoot to the base of the flower. Inflorescence stalk diameter was measured in hyacinth as the growth in girth of the stalk holding the inflorescence that is, it is a measure of thickness of the inflorescence stalk. Similarly, inflorescence diameter is a measure of inflorescence thickness before the opening of the florets and was measured as the growth in girth of the inflorescence (that is in the case of hyacinth). All these parameters were recorded using a ruler but the number of florets was recorded by counting them after they were fully opened.

2.4.3 Bulb fresh weight, gain in fresh weight and plant vigour rating

The fresh weights of bulbs were recorded with an electronic weighing balance as the weight of the bulbs prior to planting. At harvesting, gain in fresh weight was simply measured as the harvest weight less the initial fresh weight at planting. Vigour rating of plants was made by scoring the overall vigour or health of the plant stand. Considerations were given to the general plant performance and attributes such as leaf greenness, growth rate, wilting or drooping of leaves, flower development and canopy architecture, plant weakness and plant height were of paramount importance in scoring. In this measurement, a scale of 1 = very weak, 2 = weak, 3 = moderately healthy, 4 = healthy and 5 = very healthy was used. Data on scoring were log transformed prior to ANOVA, but were back transformed for easy comparison among treatment means.

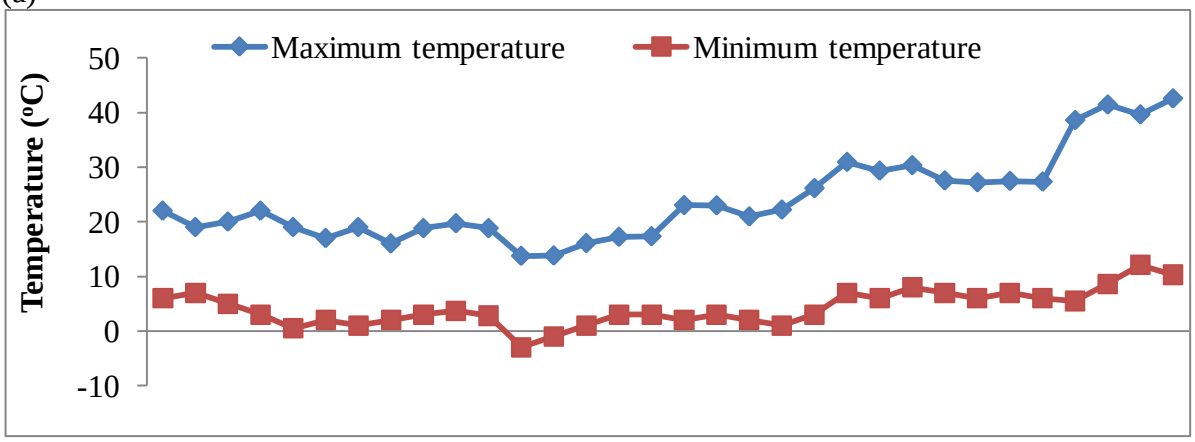
2.4.4 Chlorophyll content, photosynthetic rate and stomatal conductance

Leaf chlorophyll concentration was measured with a SPAD chlorophyll meter (Minolta SPAD-502) that gives a relative index of leaf concentration. The instrument was first calibrated and clipped to three points: the lower part, the middle portion and towards the tip of the leaf whose chlorophyll content was desired. In hyacinth, the first three leaves from outside were considered whilst in the lily, six leaves from different positions of the plant were involved: two from the lower part of the shoot, two from the middle section and two from the topmost part of the shoot system of each plant. Averages were computed for each plant prior to the analysis of this data. Using the infrared gas analyser (Ciras-1 PP Systems), photosynthetic rate and stomatal conductance were determined between the hours of 12:00 and 15:00 at the prevailing solar radiation. A known area from the leaves used for chlorophyll measurement was clipped with the cuvette of the IRGA, and measurement was made once the leaves had acclimatised to the conditions. This data was taken the same way as the chlorophyll data that is three sections of the leaves were considered for measurement.

2.5 Glasshouse conditions

The glasshouse was used for the growth of plants in all experiments as detailed under section 2.3.0. Light intensity was dependent on sunlight. Daily maximum and minimum temperature, relative humidity and light intensity were recorded between the hours of 12.00 and 14.00. Figures 2.7, 2.8, 2.9 and 2.10 show the distribution pattern of these parameters during the experimental periods.

(a)



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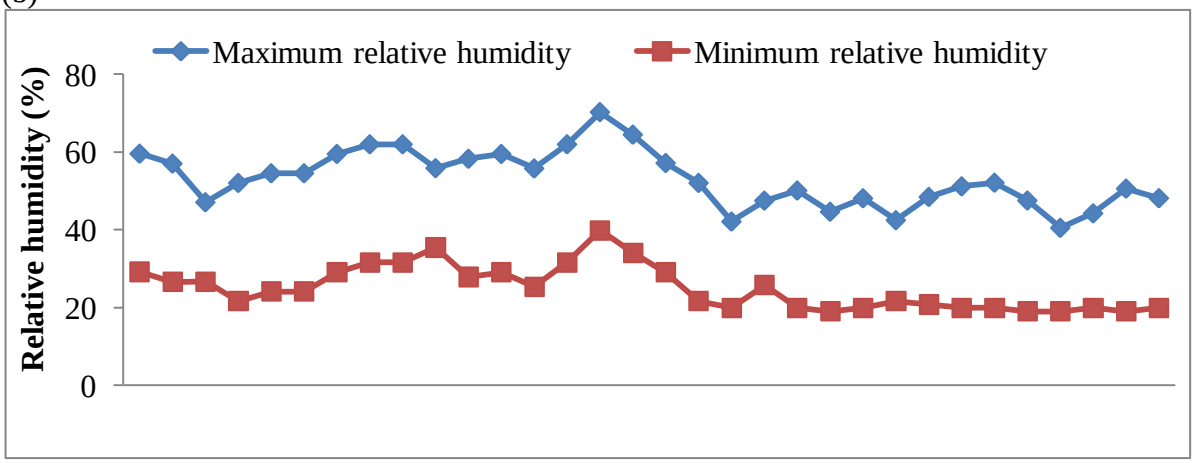

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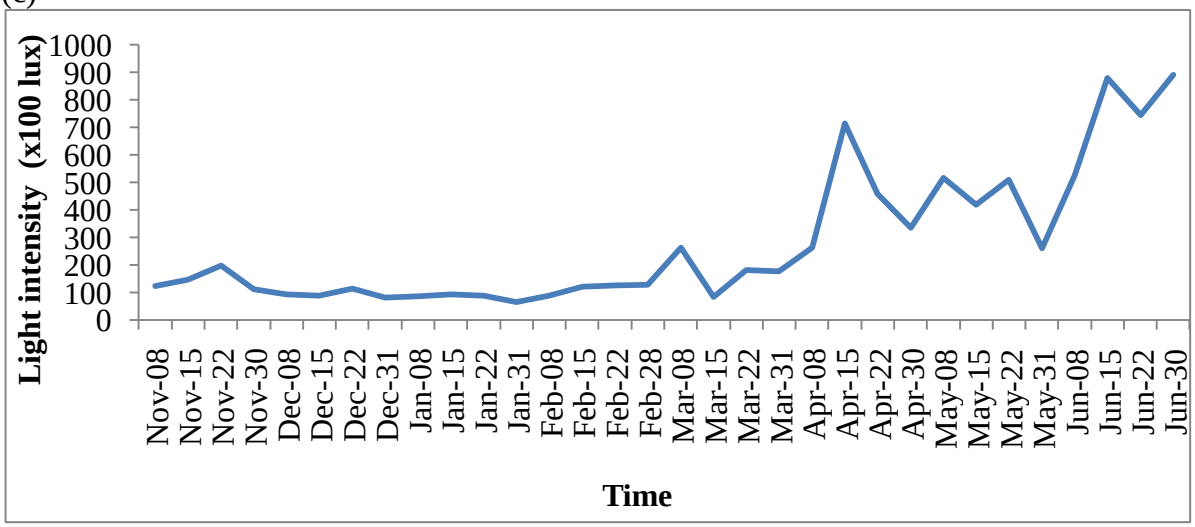


Fig 2.7: Variations in greenhouse (a) temperature (b) relative humidity and (c) light intensity from November 2006 to June 2007. Measurements were made daily in the greenhouse between the hours of 12.00 (mid-day) to 2.00 pm and averages computed for each week.

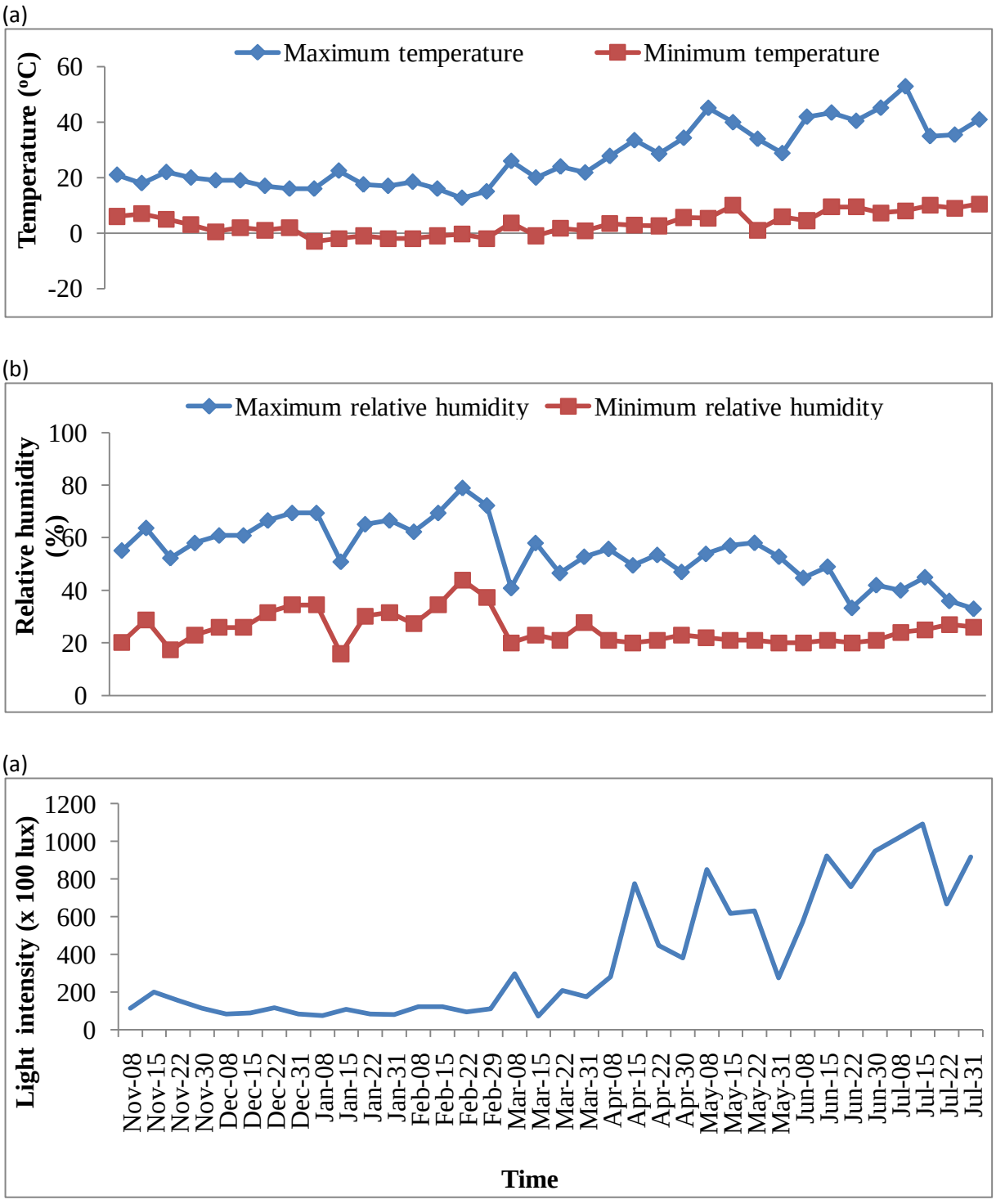


Fig 2.8: Changes in greenhouse (a) temperature (b) relative humidity and (c) light intensity from November 2007 to July 2008. Measurements were made daily in the greenhouse between the hours of 12.00 (mid-day) to 2.00 pm and averages computed for each week.

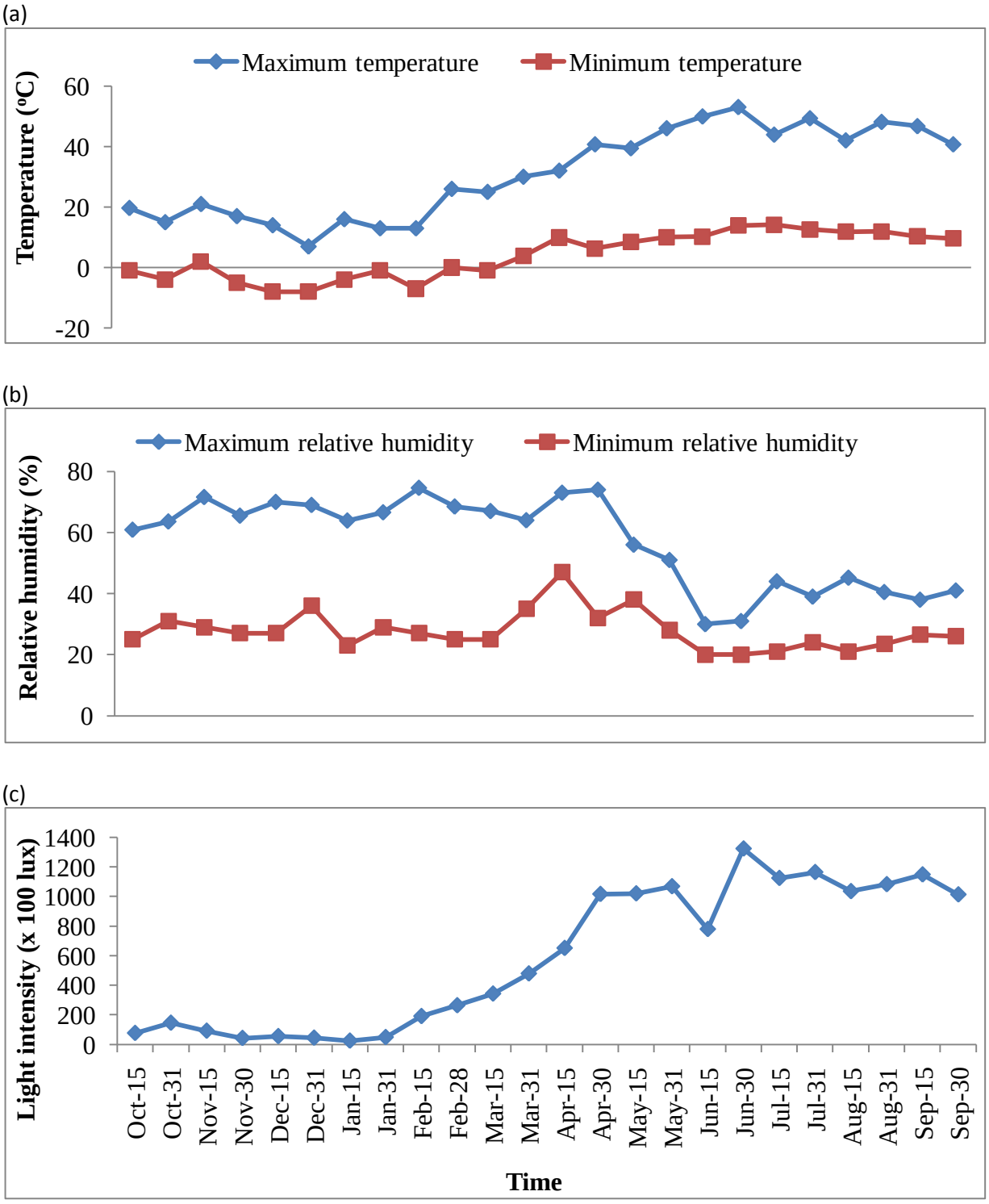


Fig 2.9: Distribution pattern of the greenhouse (a) temperature (b) relative humidity and (c) light intensity from October 2008 to September 2009. Measurements were made daily in the greenhouse between the hours of 12.00 (mid-day) to 2.00 pm and averages computed for each fortnight.

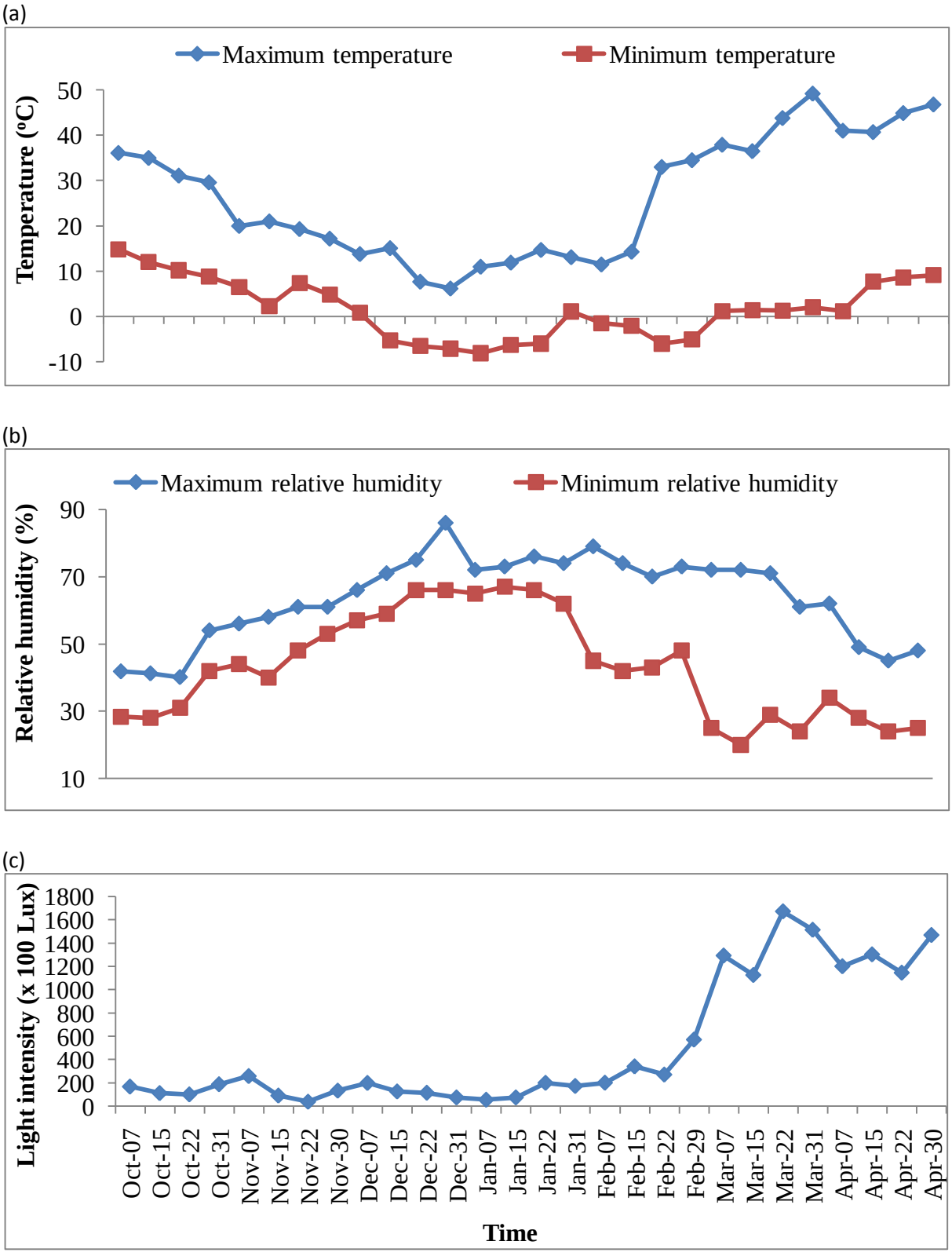


Fig 2.10: Changes in greenhouse (a) temperature (b) relative humidity and (c) light intensity from October 2009 to April 2010. Measurements were made daily in the greenhouse between the hours of 12.00 (mid-day) to 2.00 pm and averages computed for each week.

CHAPTER 3

REGULATION OF CARBOHYDRATE PARTITIONING AND METABOLISM

3.1 Introduction

The most abundant storage components in plants are the carbohydrates. These compounds are the sources of energy for plant growth and development. Carbohydrates also allow plants to synthesize many structural components as well as enhancing the distribution of energy and substrates between different tissues of the plants (Smith, 1999). It has been established that sucrose is the main translocated carbohydrate whilst starch is the major insoluble polysaccharide in plants. According to Sowokinos (2007), carbohydrate regulation and metabolism in plants may be affected by factors such as biotic and abiotic stresses during growth and development, cultivar (cultivated variety), the environment, the physiological age of the plant and enzyme activities or gene expression. These factors may not only affect the carbohydrate partitioning and metabolism of the species, but will also affect growth and development of the plant in general because partitioning and metabolism of carbohydrates has a direct influence on the source-sink relationship of plants. In Chapter 4 of this thesis, it will be known that abiotic factors such as herbivory and drought impact negatively on plant carbohydrate reserve as well as on growth and development of the study plants. Sheehan (2010) compared the carbohydrate content of orchid tubers from plants that were completely (100 %) or partially (50 %) defoliated to that of the undefoliated control and made the following observations: starch and glucose content of the tubers were higher for the control than those from the defoliated plants, the carbohydrate content of the tubers decreased as the intensity of the defoliation treatment increased and also, the tuber starch was converted into sucrose to fuel the regrowth of shoots in the case of the completely defoliated plants.

Flowering bulbs contain a number of reserve carbohydrates: glucose, fructose, sucrose, starch, glucomannans and fructans (Miller, 1992). For instance, Saniewski (1975) observed that the storage scales of hyacinth bulb contain starch, fructosans at various degrees of polymerisation, fructose, sucrose and negligible amounts of glucose. While reserve carbohydrates are known to play a vital role during the initial growth of bulbs and other geophytes, little is known about their biochemistry and metabolism (Miller, 1992). In recent years, interest and activity in the area of carbohydrate metabolism, especially information related to the regulation and partitioning of carbohydrates in geophytes has increased. The increased interest in this area of study has been attributed

to the ability of tubers, corms, roots and bulbs to store and remobilise reserved metabolites, especially carbohydrates (Miller, 1992) for their own growth and development. Though the common hyacinth (*Hyacinthus orientalis*) and the lily (*Lilium longiflorum*) are important ornamental bulbous plants cultivated mainly for indoor or outdoor decorations, very few publications on biochemistry of carbohydrate partitioning and metabolism are available on these species. Similarly, limited information is available on the degradation and biosynthesis of starch in bulbous plants. Most of the reported literature on this topic was centred on starch degradation by α-amylase in the endosperm of germinating seeds, especially in barley or rice which are all members of the *Poaceae* (Stanley *et al*., 2005). Zeeman *et al*. (2004) stated that modelling and studies involving potato (*Solanum tuberosum*) and *Arabidopsis thaliana* mutants provided the most recent understanding of starch breakdown in plants. In general, α-amylase and ADP-glucose pyrophosphorylase are the two main enzymes known to play a major role in the degradation and biosynthesis of plant starch, respectively (Beck and Ziegler, 1989; Vishnevetsky *et al*., 2000). It has been observed that α-amylase activity and size of starch grains increased within a flowering bulb from the inner to the outer scales of the bulb. The enzyme activity also increased continuously during flower bud formation and decreased during rooting (Banaski *et al*., 1980). Akazawa and Hara-Nishimura (1985) also reported that α-amylase activity increased during germination of seeds and sprouting of geophytes. Similarly, it is known that the activity of this enzyme in the storage organs of plants generally increased slightly during cold periods with the increase becoming dramatic during shoot elongation (Komiyama *et al*., 1997; Lambrechts *et al*., 1994). Since species and organs may differ in their manner of starch degradation and biosynthesis, there is the need to have adequate information on other enzymes, apart from α-amylase that also plays a role in starch metabolism in bulbous plants. Similarly, there is the need to have a good knowledge of carbohydrate content of the various organs of hyacinth and the lily at various stages of growth and development, and the distributions of the activity of enzymes related to carbohydrate metabolism in these plants. Such information will not only provide a good understanding about the physiology of these plants but also the biochemistry of carbohydrate regulation, partitioning and metabolism of the plants.

In horticultural bulb production, it has been established that, flowering bulbs lose dry weight, and the reserve stored in the bulb particularly starch also decreases after the bulb has been planted in the soil (Lambrechts *et al*., 1994; Ohyama *et al*. (1998). In *Lachenalia* cv Ronina, Du Toit *et al.* (2004) reported that the total dry weight of the bulb decreased from 1250 to 250 mg whilst starch content decreased from 90 to 10 mg/g dry weight at six weeks after planting, representing 80 and 90 % reductions of the initial values, respectively. In addition, these authors observed that the changes in dry weight of the bulb closely followed that of starch, and they explained that the depletion in starch and reserves of the bulb prior to sprouting was due to their utilisation leading to the development of newly formed leaves and roots. These observations are in agreement with that made by Wassink (1965) who also stated that the main achievements of an adult bulb plant are to build up new bulbs and that the aerial parts develop mainly at the expense of the old bulb. Chen (1969) also reported that in *Narcissus tazetta*, starch was abundantly stored in the leaf bases. The levels of hexoses were low in all parts of the dormant plant but increased with maturity, and at the time of above-ground senescence, sugars disappeared almost completely in the bulb. Also, the storage organs were depleted of carbohydrates in a centripetal manner such that the outer scales became thin and membranous as they contained only 10 % residual dry weight of starch while sugar content was below 1%. Matsuo and Mizuno (1974) also reported that the disappearance of starch in geophytes took place first in the outer scales and then, gradually, the process became inner directed during cultivation. Die *et al. (*1970) also studied translocation in bulbous plants using C^{14} labelled assimilates. They observed that the flower–bearing part of the shoot was an important sink for photosynthates, attracting materials from upper leaves of the leafy shoot as well as using its own products. The bulb also attract small quantities of assimilates from the terminal leaves, despite the proximity of the flower to this source. It is due to the above background and characteristics of flowering bulbs that the present studies sought to investigate the regulation of carbohydrates particularly starch partitioning and metabolism in hyacinth and the lily during their growth and development. The specific objectives were to determine the changes in the concentration of glucose, fructose, sucrose and starch during the growth of these plants, and also determining the relationship between starch and sugars; and the distribution of activities of hydrolytic as well as biosynthetic enzymes that are involved in starch metabolism of these bulbous species.

3.2 Results

3.2.1 Carbohydrate partitioning and metabolism of the hyacinth scales during the 2006/2007 season

Carbohydrate studies on the scales of hyacinth were conducted between November 2006 and June 2007. Bulbs were dissected before planting and at 1, 2, 3, 4, 5, 6 and 7 months after planting, into seven scales as L1, L2, L3, L4, L5, L6 and L7 (where L1 was the very outermost scales, in that order to the very innermost scale, L7). These components were used for glucose, fructose, sucrose and starch measurements (Figure 3.01 and Table 3.2). Results indicated that at time zero (before planting), fresh and dry weights as well as starch of the scales generally decreased from the very outermost to the innermost one (L1 to L7). These parameters also reduced from planting time to five months after planting and increased from five to seven months after planting (Fig 3.01a, and 3.01b; Fig 3.02a, Table 3.2). Scales L5, L6 and L7, however, increased in starch content from 2 to 3 months after planting (Fig 3.02a). The reduction in starch and dry weight of the scales was greatest during the first month after planting with the outermost scale (L1) giving greater reductions in these parameters as compared to the innermost one (L7). Thus for example at one month after planting, total starch content of the very outermost scale (L1) reduced from 1634.18 to 800.91 µmol (Fig 3.02a) representing 51 % loss in starch whereas the very innermost scale (L1) reduced in starch from 903.924 to 851.79 µmol representing 5.76 % loss in starch. Similarly, from Table 3.2a where the carbohydrates values are expressed as µmol g^{-1} fresh weight, the decrease in starch of L1 was from 306.6 to 211.6 µmol g^{-1} fresh weight, whilst L7 reduced from 214.2 to 170.7 µmol g^{-1} fresh weight of starch, representing 31 and 20%, respectively. Reductions in these quantities for L1 were similar to L2, L3 and L4, whilst those of L7 were also similar to L5 and L6. Thus scale leaves L1 to L4 displayed similar metabolic functions whilst L5 to L7 also behaved similarly. Therefore scales L1 – L4 were grouped together as outer scales whilst L5, L6 and L7 were also classified as inner scales for further studies during the 2007/2008 planting season. The 2006/2007 study also revealed that moisture content (Fig 3.01c) of the scales increased from planting time to four months after planting and decreased from this point to seven months after planting. The soluble sugars: glucose, fructose and sucrose (Fig 3.02b, c, d; Table 3.2b, c, d) also decreased from planting time to two months after planting, increased from two to five months after planting and almost disappeared at seven

months after planting. That is, whereas starch accumulated in the scales from five to seven months after planting, the sugars almost disappeared from the scales within this period. The disappearance of sugars from the scales as starch accumulated towards the end of the season is also shown by the correlation of starch and soluble sugars content of the scales as found in Table 3.1: r = -0.094, -0.276, -0.046 for glucose, fructose and sucrose, respectively. The higher values of starch of the scales compared to the very low amounts of the sugars measured (Table 3.2), especially prior to planting of the bulbs, coupled with the degradation of starch which resulted in the general decrease of the polysaccharide from time zero to five months after planting of the scales, and the accumulation of starch or the disappearance of sugars towards the end of the growing season, implies that starch was the major storage carbohydrate in the scales of hyacinth bulb.

Table 3.1: Correlation matrix of biomass and the various carbohydrates of the hyacinth scales

	Fresh weight	Dry weight	Starch	Glucose	Fructose	Sucrose
Fresh weight		0.869	0.669	0.562	-0.244	0.519
Dry weight			0.694	0.611	-0.155	0.327
Starch				-0.094	-0.276	-0.046
Glucose					0.563	0.925
Fructose						0.520
Sucrose						

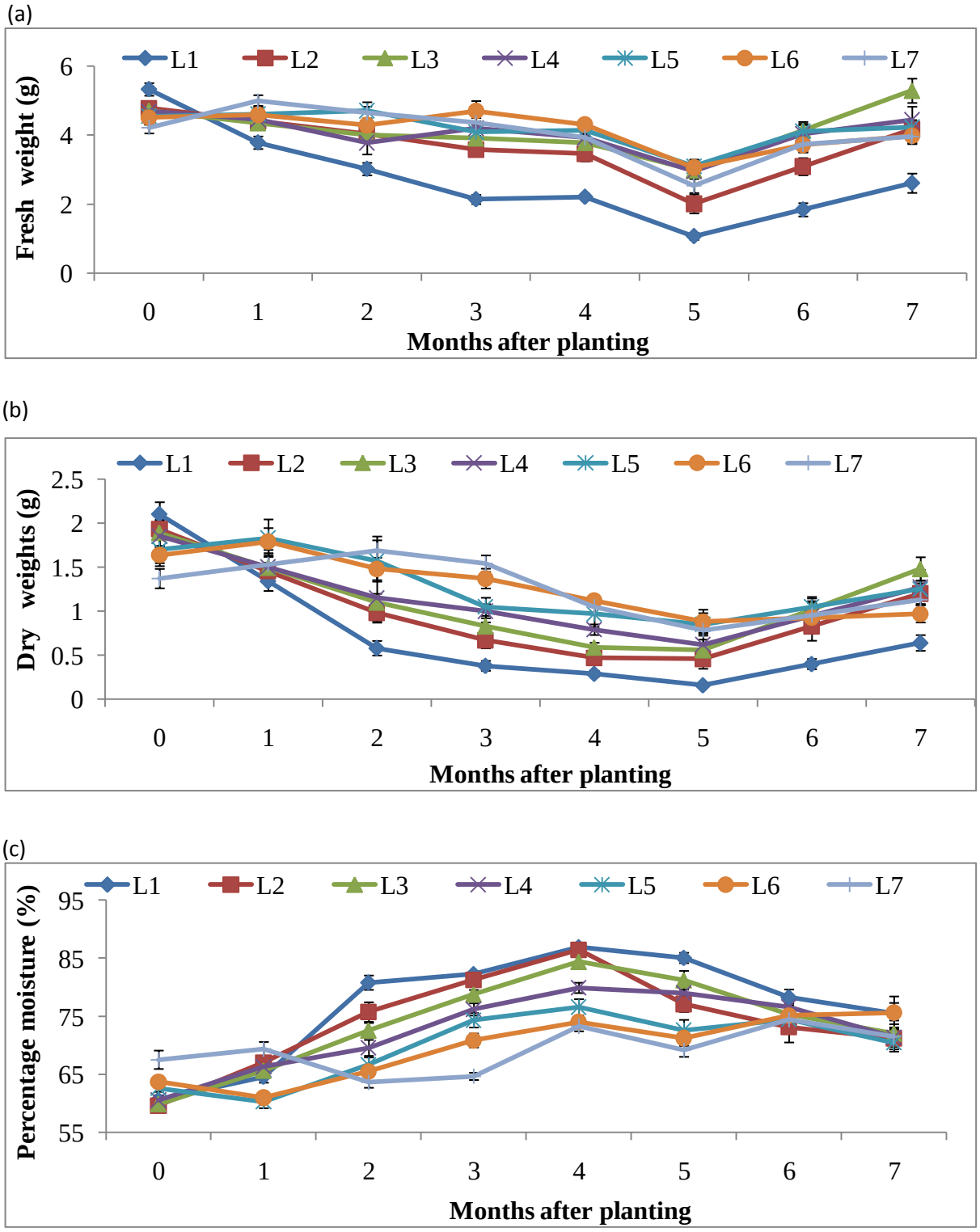


Fig 3.01: Changes in (a) fresh weight, (b) dry weight and (c) moisture content of hyacinth scales during the growth of the bulb in 2006/2007 season. Bars represent means ± SE of five replicates.

Table 3.2a: Carbohydrate content of the scales of hyacinth during the growth of the bulb in 2006/2007: (a) starch, (b) glucose. Values in this table are expressed as µmol g^{-1} fresh weight.

(a)

	Months after planting							
Scale leaves	0	1	2	3	4	5	6	7
L1	306.6 ± 52	211.6 ± 34	116.6 ± 15	71.8 ± 14	38.5 ± 4	12.1 ± 13	109.2 ± 15	450 ± 16
L2	284.4 ± 40	209.5 ± 27	134.7 ± 14	94 .0 ± 50	51.6 ± 9	16.8 ± 21	148.5 ± 16	432.1 ± 12
L3	281.6 ± 54	204.2 ± 30	126.7 ± 60	96.1 ± 90	53.2 ± 12	15.9 ± 26	143.2 ± 21	377.0 ± 17
L4	255.6 ± 35	203.7 ± 25	151.7 ± 16	117.4 ± 16	98.3 ± 10	32.9 ± 70	148.5 ± 11	372.5 ± 16
L5	249.5 ± 27	172.6 ± 24	95.6 ± 21	122.6 ± 22	88.5 ± 10	26.6 ± 13	126.4 ± 10	273.6 ± 70
L6	234.3 ± 47	171.6 ± 33	108.9 ± 18	159.7 ± 80	86.2 ± 90	25.9 ± 30	144.0 ± 50	273.5 ± 70
L7	214.2 ± 26	170.7 ± 18	127.2 ± 10	165.9 ± 13	80.6 ± 70	23.2 ± 70	141.6 ± 70	278.8 ± 70

(b)

L1	11.8 ± 1.5	9.4 ± 1.2	6.9 ± 0.9	4.9 ± 1.1	9.9 ± 2.3	4.0 ± 0.8	3.4 ± 1.2	2.8 ± 1.6
L2	12.6 ±1.1	8.9 ± 0.9	5.2 ± 0.8	7.3 ± 2.5	8.1 ± 2.4	6.3 ± 0.7	4.6 ± 1.3	2.9 ± 1.8
L3	12.5 ± 1.2	8.7 ± 0.8	4.9 ± 0.4	8.3 ± 1.1	11.3± 3.0	4.7 ± 0.6	3.6 ± 1.1	2.5 ± 1.6
L4	14.3 ± 1.3	9.7 ± 1.1	5.2 ± 0.9	9.9 ± 0.7	9.5 ± 1.6	7.1 ± 0.9	4.0 ± 0.6	1.0 ± 0.3
L5	12.3 ± 1.7	8.6 ± 1.0	4.9 ±0.4	9.5 ± 0.4	13.5 ± 2.0	6.0 ± 0.3	3.2 ± 0.2	0.4 ± 0.1
L6	13.4 ± 1.0	9.7 ± 0.8	6.1 ± 0.5	11.5 ± 1.8	10.8 ± 1.6	7.0 ± 0.8	3.6 ± 0.5	0.2 ± 0.1
L7	11.8 ± 1.2	8.6 ± 1.0	5.4 ± 0.8	13.5 ± 1.1	11.7 ±1.1	1.9 ± 1.0	0.9 ± 0.7	0.1 ± 0.3

Table 3.2b: Carbohydrate content of the scales of hyacinth during the growth of the bulb in 2006/2007: (c) fructose, and (d) sucrose. Values in this table are expressed as µmol g^{-1} fresh weight.

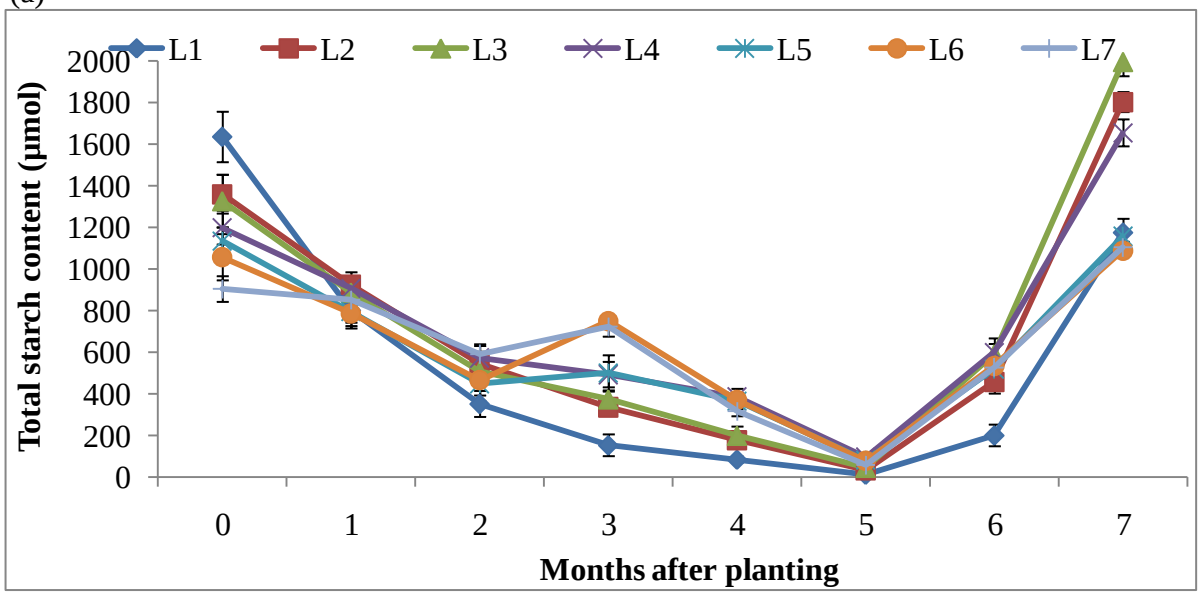
(c)

	Months after planting							
Scale leaves	0	1	2	3	4	5	6	7
L1	4.4 ± 1.4	2.8 ± 1.0	1.1 ± 0.6	4.7 ± 0.8	2.8 ± 0.2	4.0 ± 0.3	2.7 ± 0.6	1.5 ± 0.8
L2	4.9 ± 0.7	2.8 ± 0.5	0.6 ± 0.3	1.6 ± 0.4	3.9 ± 1.4	1.7 ± 1.4	1.6 ± 1.1	1.6 ± 0.7
L3	2.6 ± 0.6	1.8 ± 0.4	0.9 ±0.2	2.1 ± 0.2	4.1 ± 2.1	0.1 ± 2.3	0.2 ± 1.3	0.3 ± 0.3
L4	3.2 ± 0.6	1.7 ± 0.5	0.1± 0.4	1.2 ± 0.5	5.1 ± 1.0	3.7 ± 2.5	1.9 ± 2.3	0.2 ± 0.1
L5	4.3 ± 1.1	2.5 ± 0.7	0.7 ± 0.3	1.6 ± 0.4	3.2 ± 0.5	2.8 ± 1.2	1.4 ± 0.6	0.2 ± 0.1
L6	0.9 ± 0.5	0.8 ± 0.4	0.7 ± 0.3	0.3 ± 0.7	4.8 ± 1.2	4.6 ± 1.4	2.3 ± 0.7	0.2 ± 0.1
L7	0.7 ± 0.8	0.7 ± 0.6	0.7 ±0.4	0.3 ± 0.1	4.0 ± 0.9	2.8 ± 1.9	1.5 ± 1.0	0.3 ± 0.1

(d)

L1	16.3 ± 2.1	12.1 ± 1.6	8.0 ± 1.1	9.6 ± 1.6	12.7 ± 2.4	8.0 ± 0.6	6.2 ±1.2	4.3 ± 1.8
L2	17.4 ± 1.0	11.7 ± 0.9	5.9 ± 0.8	8.9 ± 2.2	11.9 ± 3.8	4.7 ± 2.1	4.6± 2.2	4.5 ± 2.3
L3	15.1 ± 1.6	10.5 ± 1.0	5.8 ± 0.4	10.3 ± 1.2	15.4 ± 4.9	4.6 ± 1.7	3.3 ± 1.8	2.0 ± 1.9
L4	17.5 ± 1.7	11.8 ± 1.4	6.0 ± 1.1	11.0 ± 0.5	14.6 ± 2.1	3.4 ± 3.6	2.1 ± 2.0	0.9 ± 0.4
L5	14.6 ± 2.4	10.1 ± 1.5	5.6 ± 0.5	11.1 ± 0.7	16.7 ± 1.6	3.2 ± 1.0	1.8 ± 0.5	0.4 ± 0.1
L6	14.1 ± 0.7	10.4 ± 0.5	6.7 ± 0.3	11.3 ± 1.7	15.6 ± 1.0	2.5 ± 0.7	1.3 ± 0.4	0.1 ± 0.1
L7	12.5 ± 1.1	9.3 ± 1.0	6.0 ± 0.9	13.8 ± 1.0	15.7 ± 1.0	4.6 ± 2.3	2.4 ± 0.1	0.2 ± 0.2

(a)



(b)

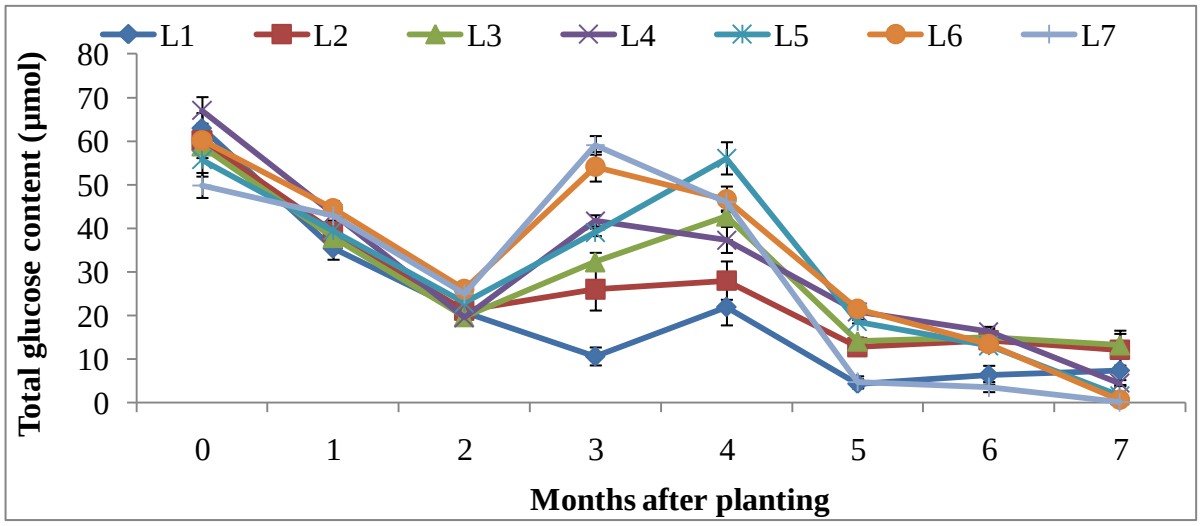
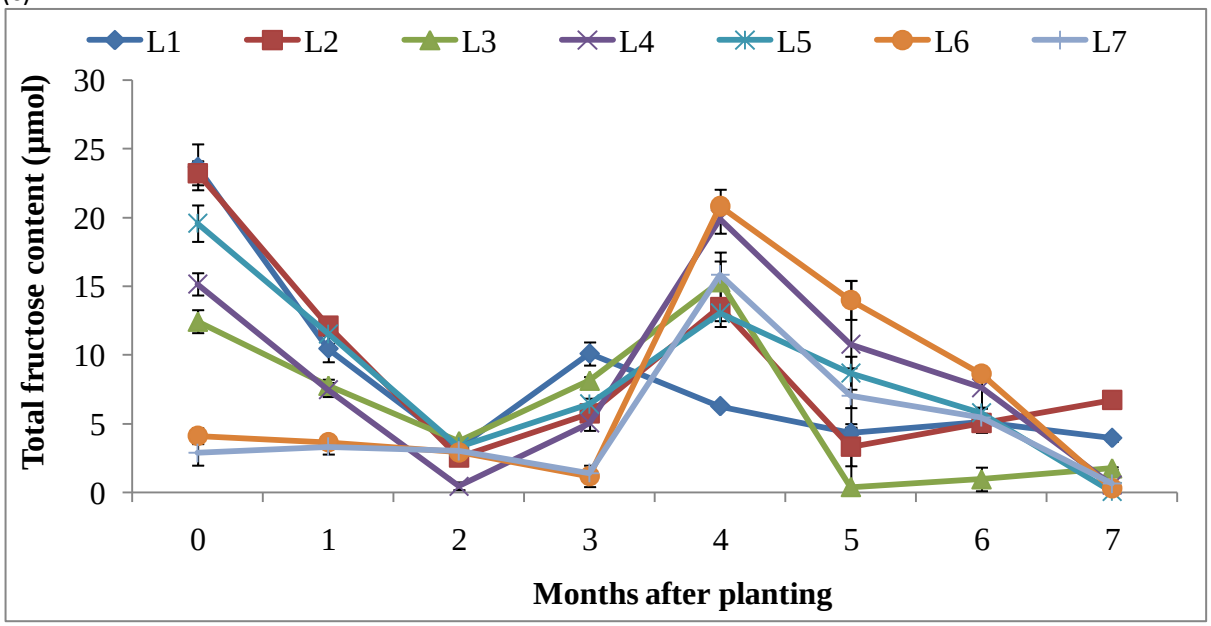


Fig 3.02a: Variation in carbohydrate concentration of hyacinth scales during the 2006/2007 planting season; total starch (a), total glucose (b). Bars represent means ± SE of five replicates.

(c)



(d)

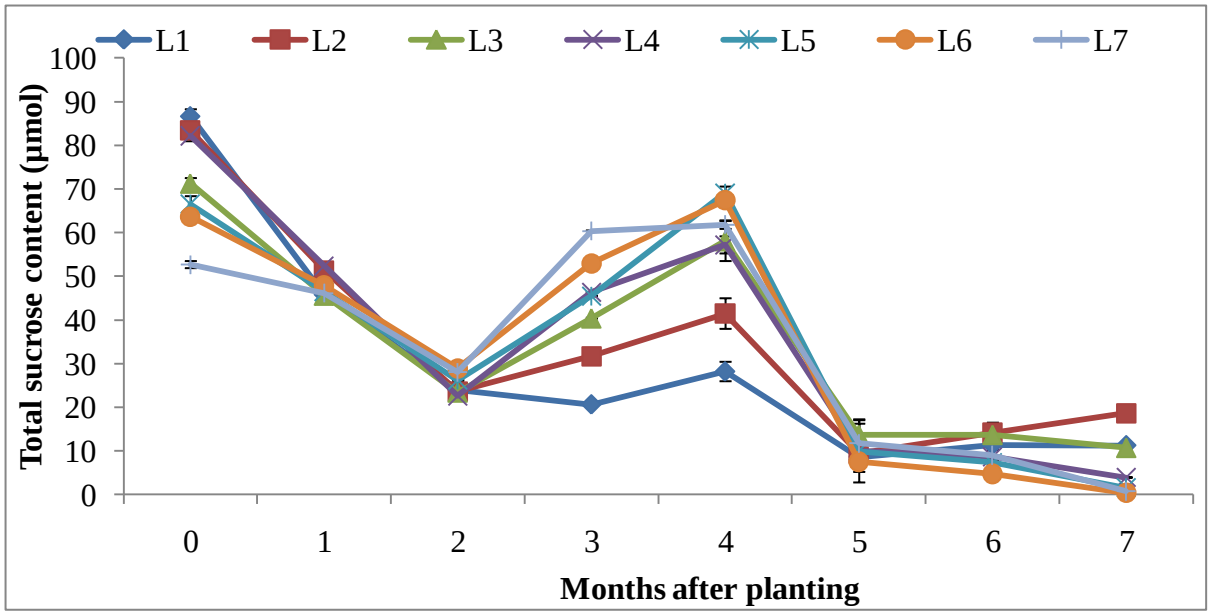


Fig 3.02b: Variation in carbohydrate concentration of hyacinth scales during the 2006/2007 planting season; total fructose (c), and total sucrose (d). Values of the various carbohydrates were obtained by multiplying the corresponding fresh weight of the tissues (Fig 3.2a) by the carbohydrate values in Table 3.1. Bars represent means ± SE of five replicates.

3.2.2 Starch metabolism of hyacinth during bulb production in the 2007/2008 season

Results of carbohydrate studies of hyacinth scales during the 2006/2007 year revealed that the starch content of the scales ranged from 306.6 to 214.2 µmol g^{-1} fresh weight whilst glucose, fructose and sucrose levels ranged from 14.3-11.8, 4.9-0.7 and 17.4-12.5 µmol g^{-1} fresh weight, respectively (Table 3.2a, b, c, d). The sugar levels of the scales generally decreased and almost disappeared especially from 4 to 7 months but prior to harvesting, there was an accumulation of starch in the scales (Fig 3.02). This implies that starch was the major storage carbohydrate in the bulb. Also scales L1-L4 behaved similarly whilst L5-L7 also exhibited similar metabolic functions. Therefore between October 2007 and May 2008, hyacinth bulbs of similar fresh weight as those from the previous study were planted and dissected into outer scales (L1–L4), inner scales (L5-L7), newly formed leaves, stem (basal plate) plus roots and flower for starch measurement at 0, 2, 4, 6, 8, 10, 12, 14 and 16 weeks after planting. Results from this experiment showed that biomass and starch content of the scales mimicked those of the previous year; that is, fresh and dry weights as well as starch of the scales were higher in outermost scales than the inner scales prior to planting (Fig 3.03a and c; Fig 3.04b). These parameters also decreased from planting to sixteen weeks after planting with the outer scales recording higher reductions in these parameters than the inner ones particularly from 0 to 4 weeks (one month) after planting as was observed in the 2006/2007 season. For instance, the total starch content of the outer scales decreased from 7713.15 to 4820.17 µmol at 4 weeks after planting (Fig 3.04b), whilst the inner scales reduced in starch from 5580.11 to 4628.11 µmol, representing reductions of 38 and 17 %, respectively. However, the newly formed organs: stem and roots, new leaves, and flowers showed an increasing trend in these quantities (Fig 3.03b and d; Fig 3.04c). The increase in starch and biomass of the flower was gradual from zero to ten weeks after planting, but very pronounced from ten to fourteen weeks after planting. Also, biomass and starch values of the stem and roots as well as those of the flowers decreased from twelve to sixteen weeks after planting. Moisture content of the organs generally increased from planting to 16 weeks after planting (Fig 3.04a).

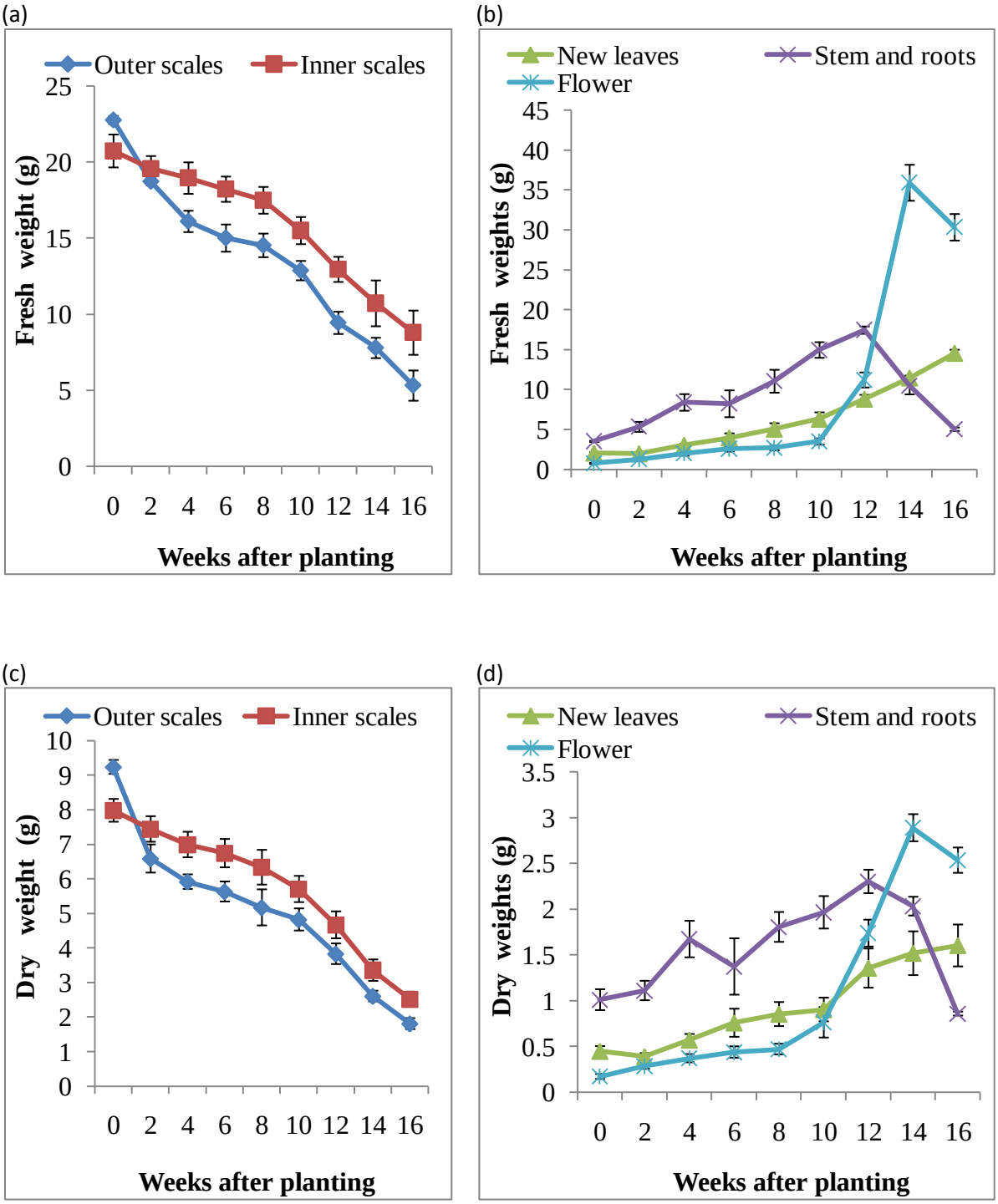
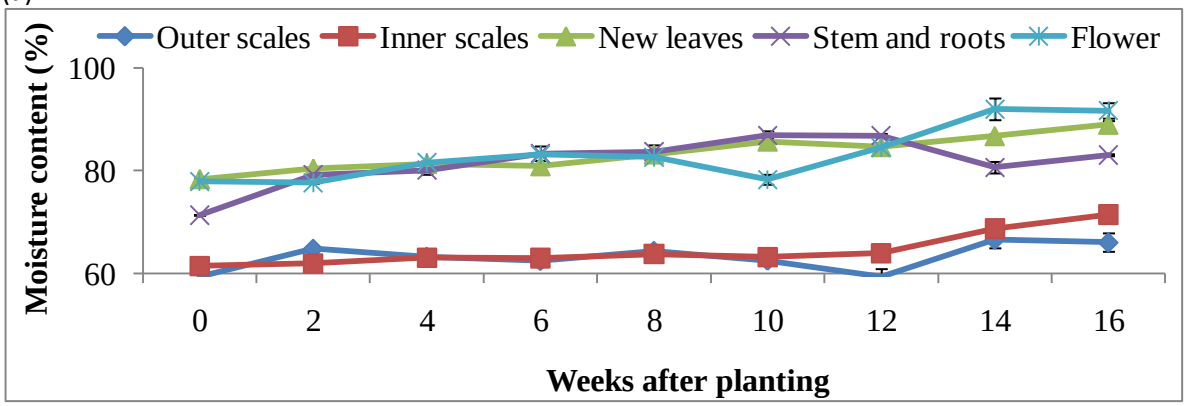
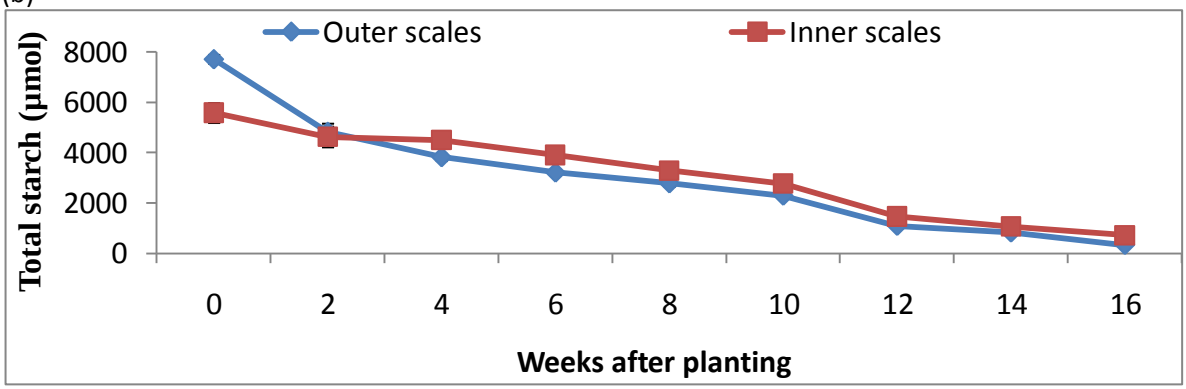


Fig 3.03: Variation in biomass of hyacinth organs; fresh weight of outer scales (a), fresh weight of the newly formed leaves, stem and roots and flower (b), dry weight of the outer and inner scales (c) and dry weight of the new leaves, stem and roots, and the flower (d). Bars represent means ± SE of five replicates.

(a)



(b)



(c)

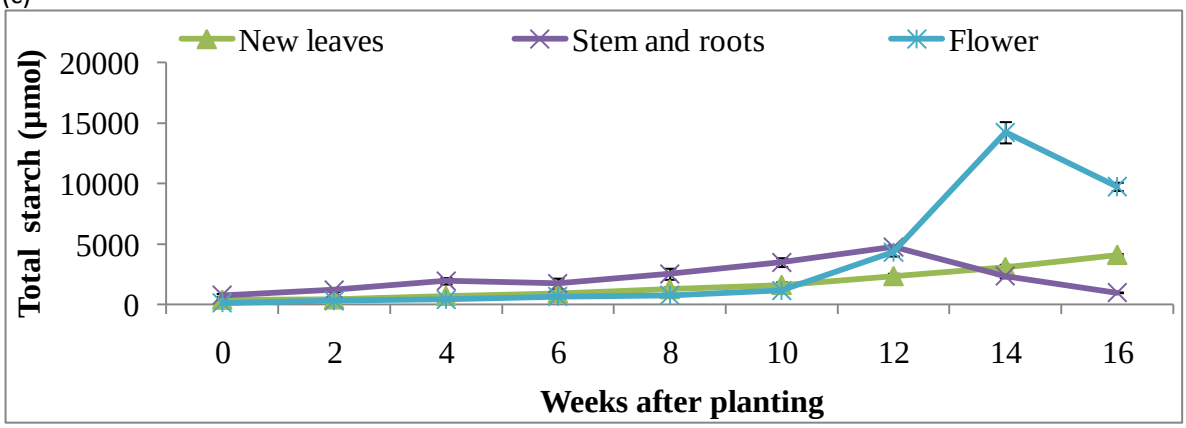
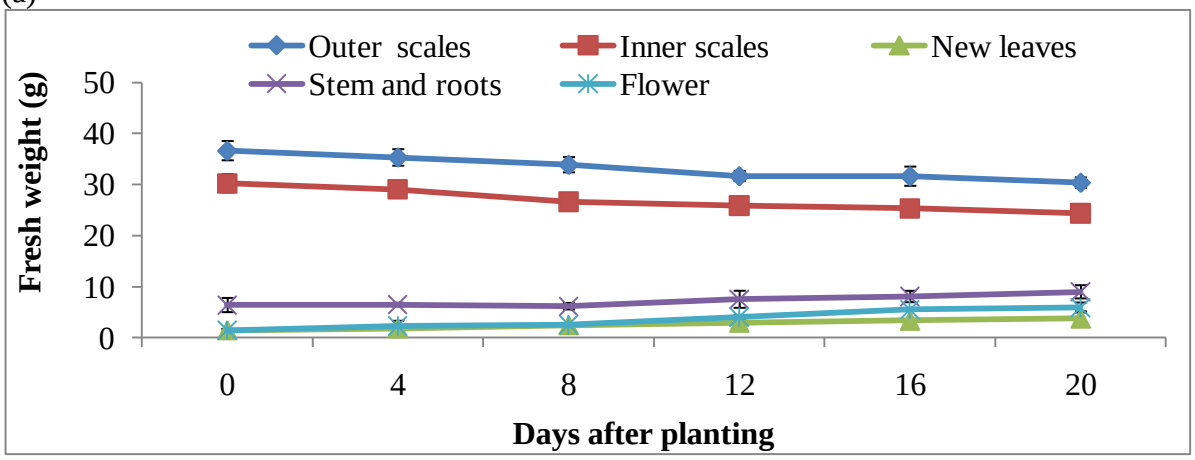


Fig 3.04: Changes in moisture and starch content of hyacinth bulb organs. The figure indicates the variation in moisture content of the organs (a), total starch content of the outer and inner scales (b) and total starch concentration of the new leaves, stem and roots, and the flower (c). Bars represent means ± SE of five replicates.

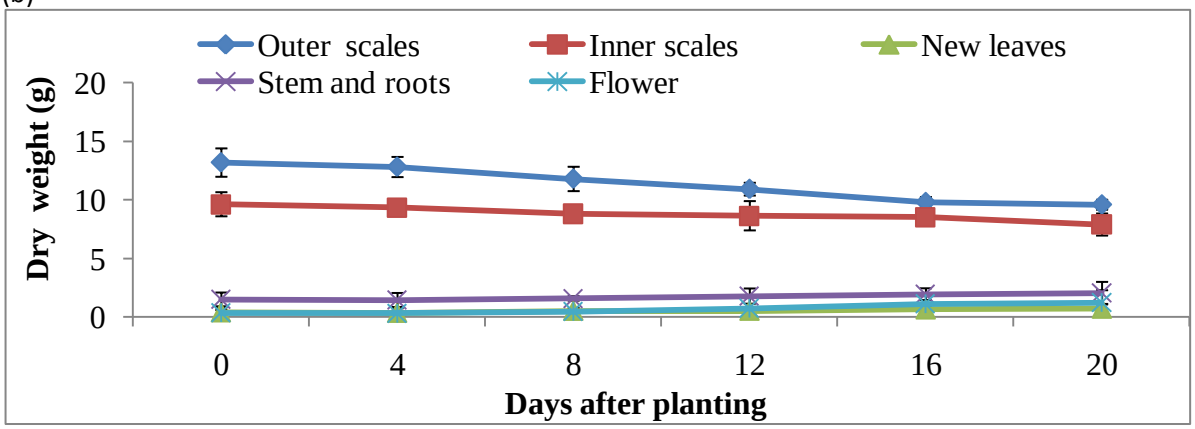
3.2.3 Starch metabolism of hyacinth when the bulbs were planted either in the soil or in glass tubes during the 2008/2009 planting season.

Results from the previous studies on starch metabolism in hyacinth indicated that the bulb lost a major percentage of its starch, especially from the outer scales during the first month after planting. The very high percentage of starch lost from the bulb scales during the early developmental phases of the bulb was assumed to be the result of either a leakage of carbohydrate from the scales, particularly from the outer scales, or as a result of the rapid emergence of shoots and roots that characterised planting of bulbs in the soil. The latter hypothesis was true when bulbs of this species were planted at 0 cm depth because sprouting of the bulbs that were planted at 0 cm depth delayed, as compared to those bulbs that were planted at 5 cm depth (see Chapter 5 for details). The study therefore sought to determine an alternative method of planting hyacinth bulbs with a view to reducing this loss in starch and biomass during the early stages of the bulb development. Planting of the bulbs in glass tubes (hydroponics) was one of the immediate options that came in mind because, among the factors known to affect carbohydrate partitioning and metabolism in plants, is the immediate environment in which the plant grows (Sowokinos, 2007). Studies were thus conducted between October 2008 and March 2009 to investigate this loss in starch and biomass that occurred during the early developmental phase of the bulb after planting. In this study, the bulbs were planted both in the soil and glass tubes for 20 days, and the starch content of the outer scales, inner scales, new leaves, stem and roots and the flower monitored every four days, starting from time zero. Results from this experiment revealed a trend that was similar to those from the previous studies (Fig 3.05- 3.08). However, in general, bulbs planted in glass tubes recorded lower percentage reductions in biomass and starch than those planted in the soil (Fig 3.09a, b, and c) indicating that planting in tubes offered the opportunity to minimise the high reductions in starch and biomass that characterised the early stages of the bulbs when planted in the soil.

(a)



(b)



(c)

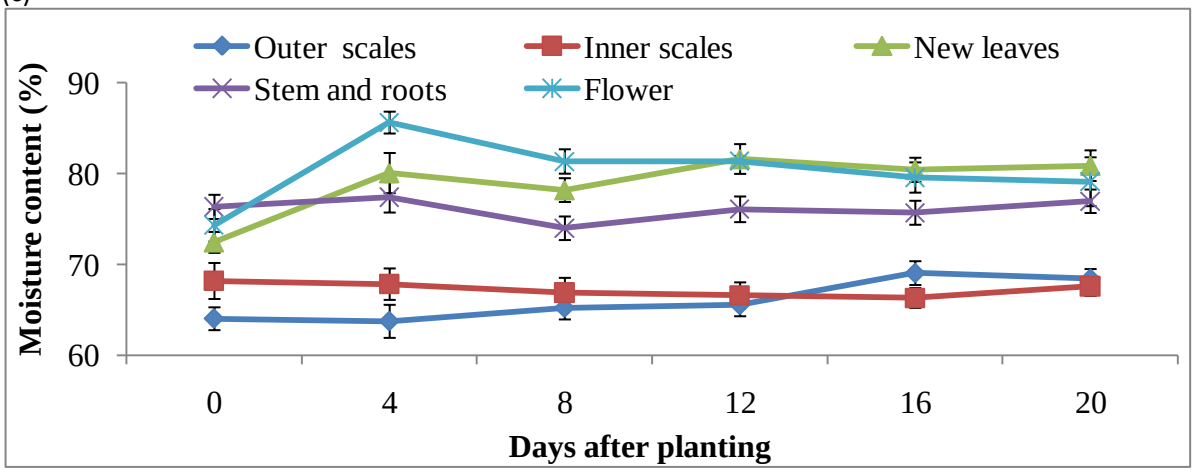
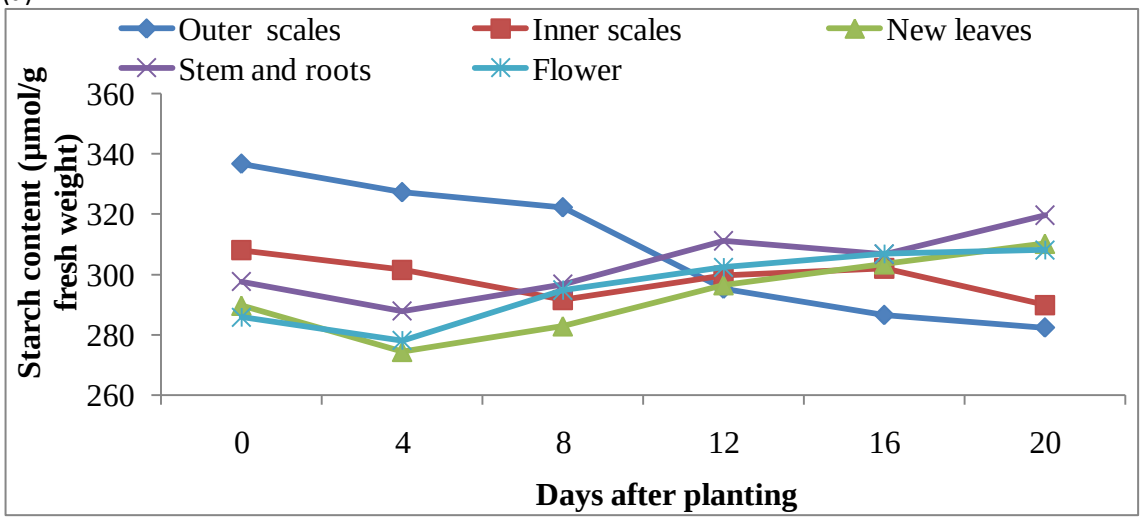


Fig 3.05: Variation in fresh weight (a), dry weight (b) and moisture content (c) of hyacinth organs during the 20 days period when bulbs were planted in the soil. Bars represent means ± SE of five replicates.

(a)



(b)

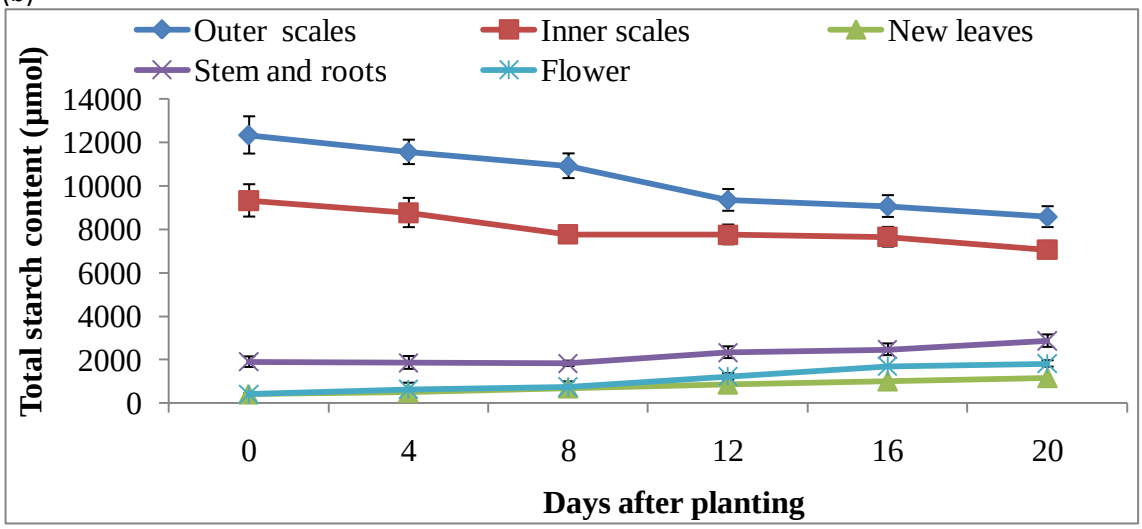
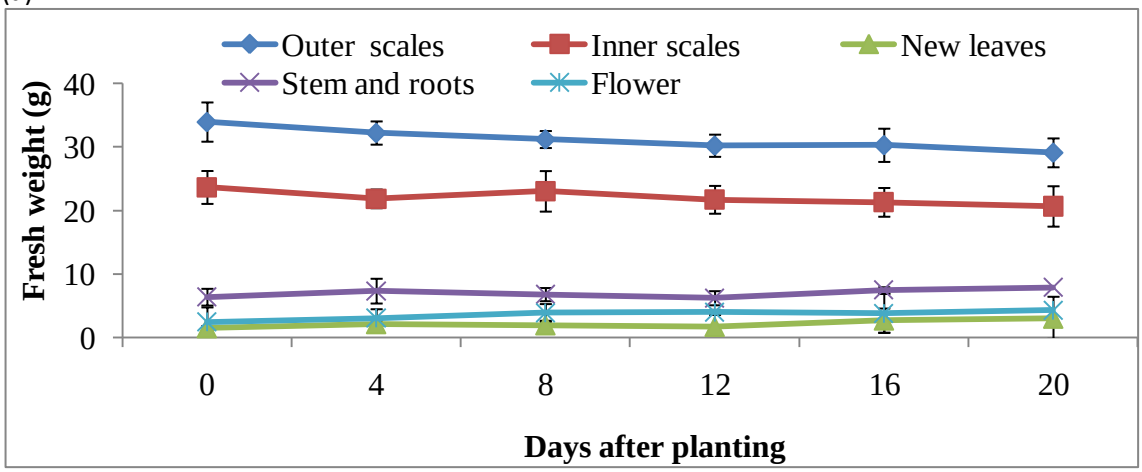
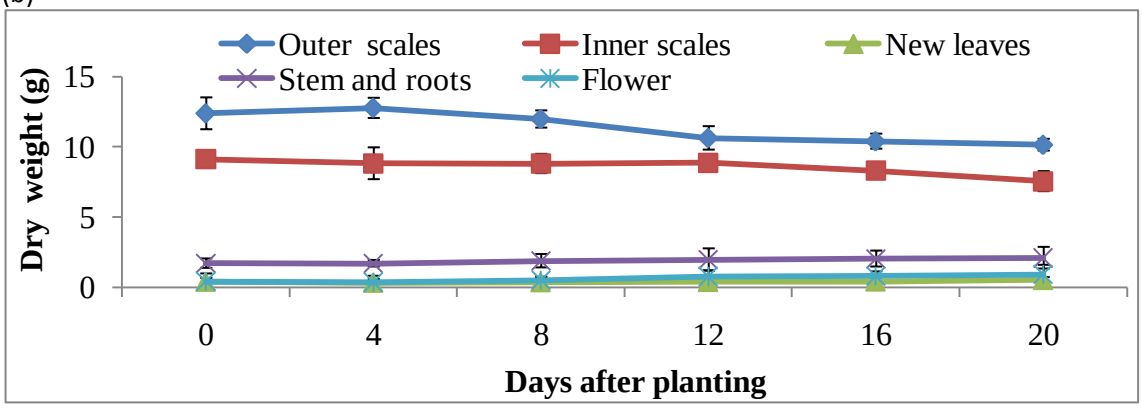


Fig 3.06: Variation in starch content of hyacinth organs during the 20 days period when bulbs were planted in the soil: (a) starch in µmol g^{-1} fresh weight of the organ, (b) and total starch in each organ. Bars represent means ± SE of five replicates.

(a)



(b)



(c)

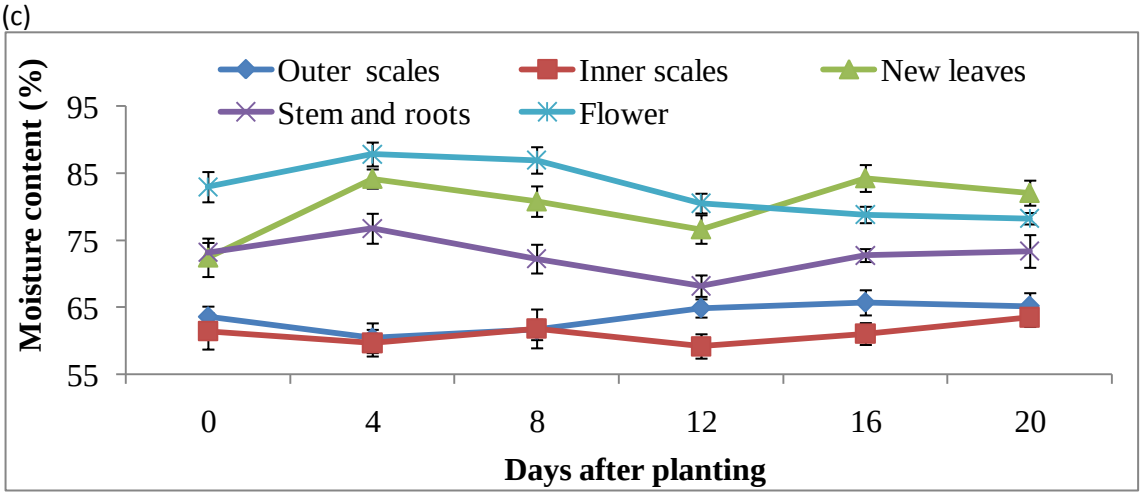
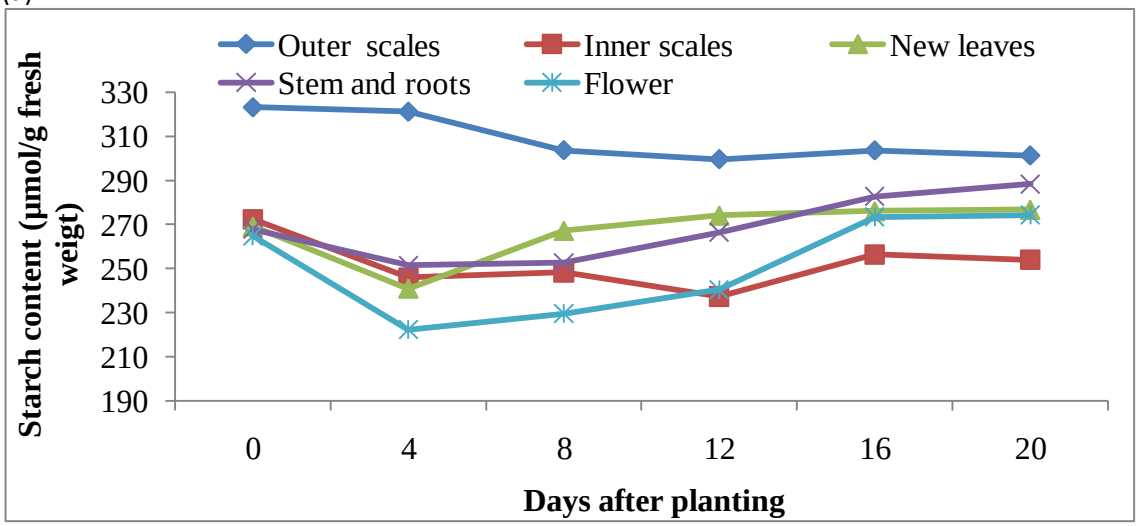


Fig 3.07: Variation in (a) fresh weight, (b) dry weight, and (c) moisture content of hyacinth organs during the 20 days period when bulbs were planted in glass tubes. Bars represent means ± SE of five replicates.

(a)



(b)

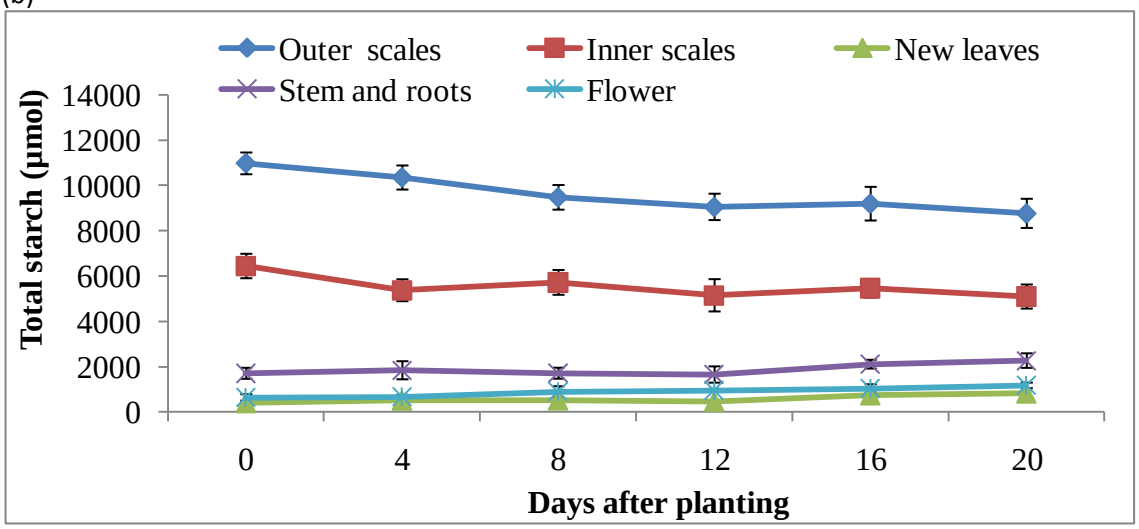
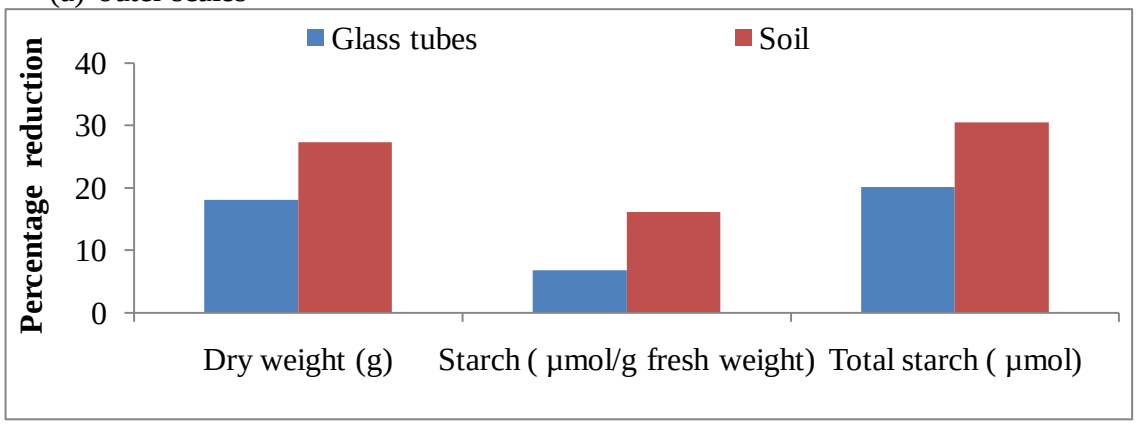
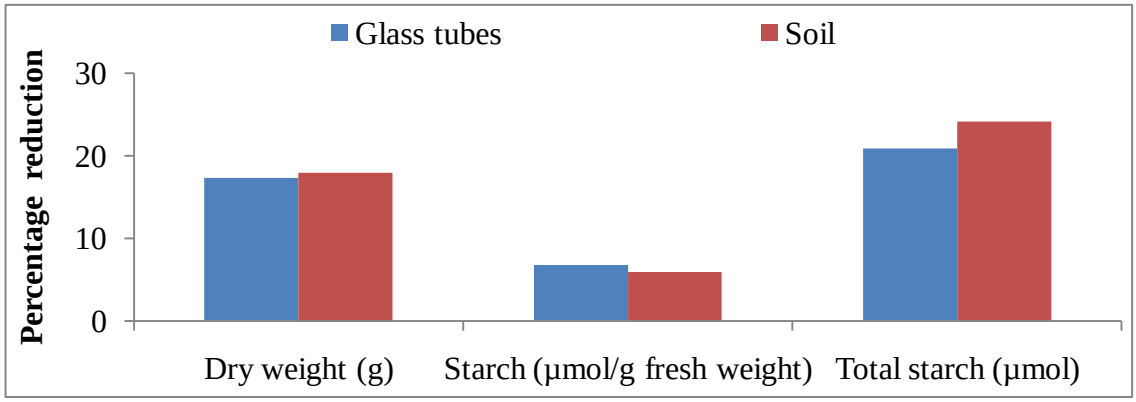


Fig 3.08: Variation in starch content of hyacinth organs during the 20 days period when bulbs were planted in the glass tubes: (a) starch in µmol g $^{-1}$ fresh weight of the organ, and (b) total starch in each organ. Bars represent means ± SE of five replicates.

(a) outer scales



(a) inner scales



(b) whole bulb

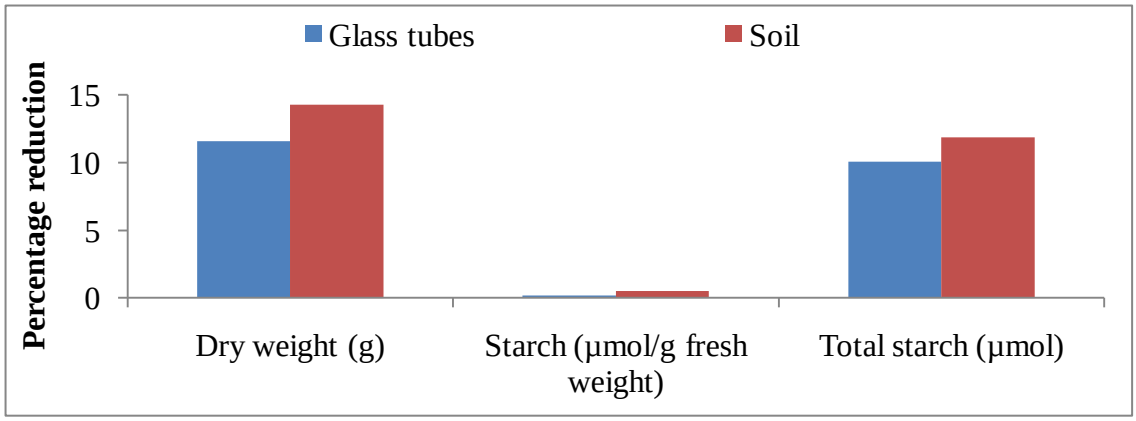
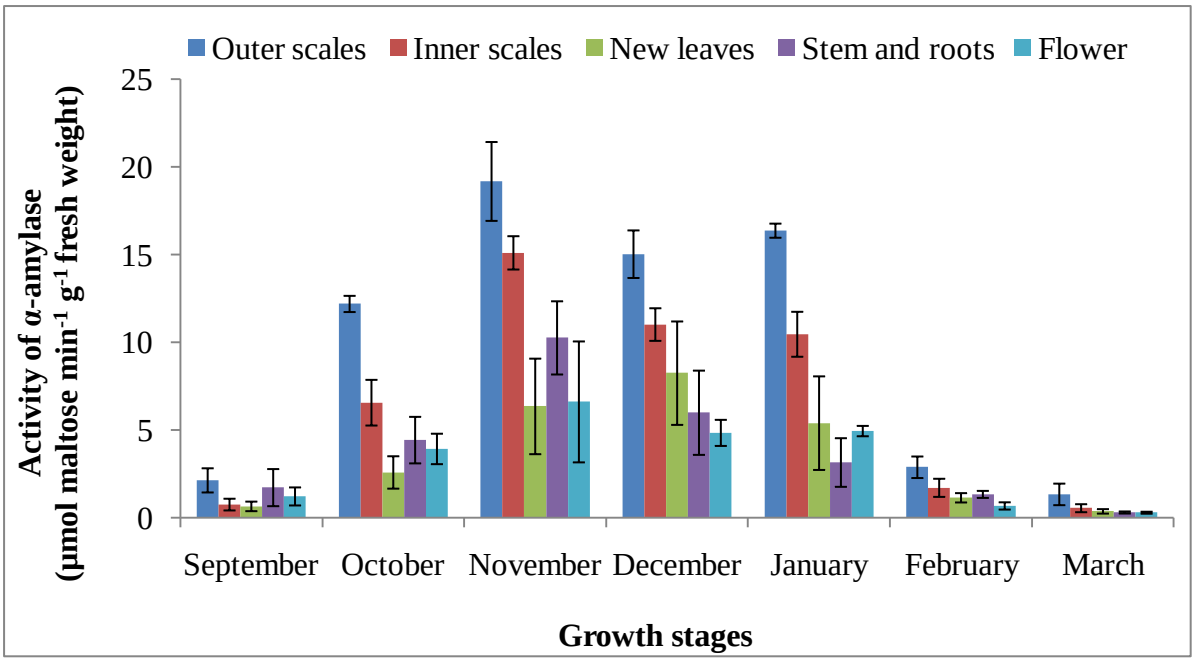


Fig 3.09: Percentage reduction in starch and biomass of the outer scales (a), inner scales (b), and the complete bulb (c), when bulbs were planted either in the soil or glass tubes. Bars represent means ± SE of five replicates.

3.2.4 Starch metabolism and distribution of enzymes activity of the hyacinth bulb

Sowokinos (2007) reported that enzyme activity or gene expression of enzymes involved in carbohydrate metabolism influences the process of regulation and partitioning of carbohydrates in plants. Therefore as part of this study, measurements of activity of some enzymes that play a major role in starch biosynthesis and degradation of hyacinth were made between September 2009 and March 2010. Bulbs were planted in September and measurements were carried out every month starting from September (before planting) to March (six month after planting). The activity of these enzymes was correlated with the starch measurements carried out in 2008/2009 season. Results indicated that the distribution of both α-amylase and starch phosphorylase enzymes were significantly different in the hyacinth scales, with the outer scales having higher activity of these enzymes than the inner scales. In general, the activity of these enzymes increased from the inside of the bulb to the outermost scales (Fig 3.10). Alpha amylase activity increased from planting to November coinciding with the period that starch degradation was at its peak in hyacinth, then decreased from December to March; however, the enzyme activity remains the same from December to January. Starch phosphorylase activity rather increased from September to October (one month after planting) and decreased thereafter to March. The correlation studies (Fig 3.12-3.13) revealed that α-amylase better related with starch degradation especially in the outer scales than starch phosphorylase (Fig 3.12) because the former correlated more positively (Fig 3.12) with starch degradation in the scales of the bulb than did the latter (Fig 3.13). Also the activity of ADP glucose pyrophosphorylase of the scales particularly that of the outer scales decreased from September to January and rose to March whilst the activity of the same enzyme in the flower, new leaves and stem generally increased from planting (September) to January (four months after planting), mimicking the distribution depicted by starch content of these organs. The distribution shown by starch synthase was similar to that of ADP glucose pyrophosphorylase but these two enzymes correlated better with starch accumulation of the flower and the newly formed leaves than they did with the stem and roots (Fig 3.14 and 3.15).

(a)



(b)

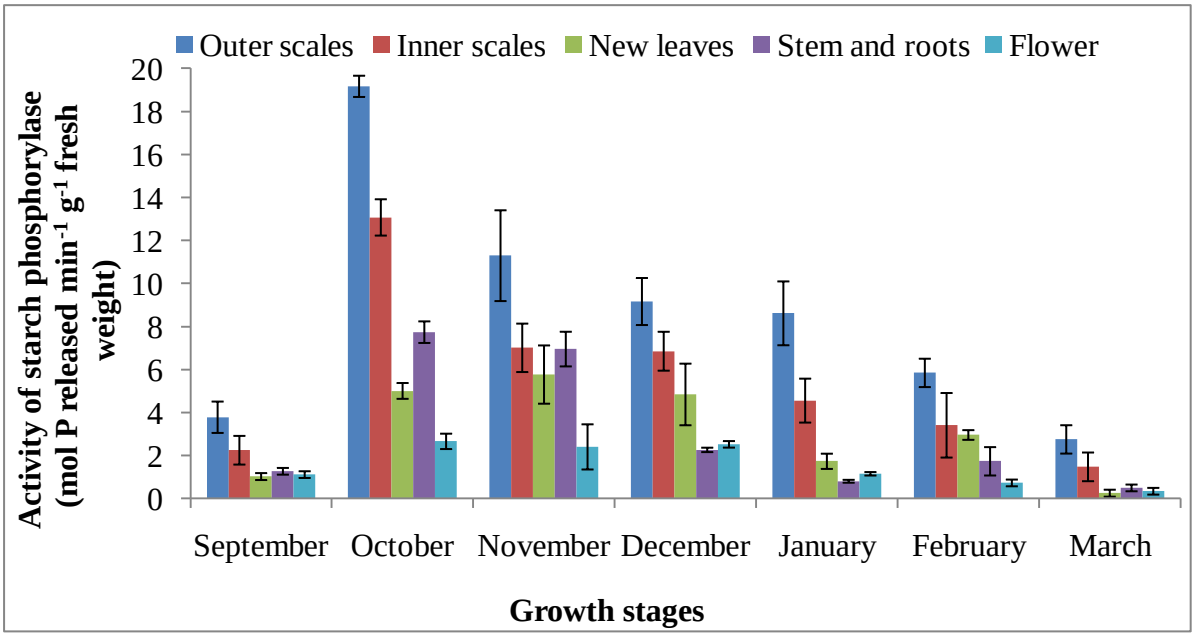
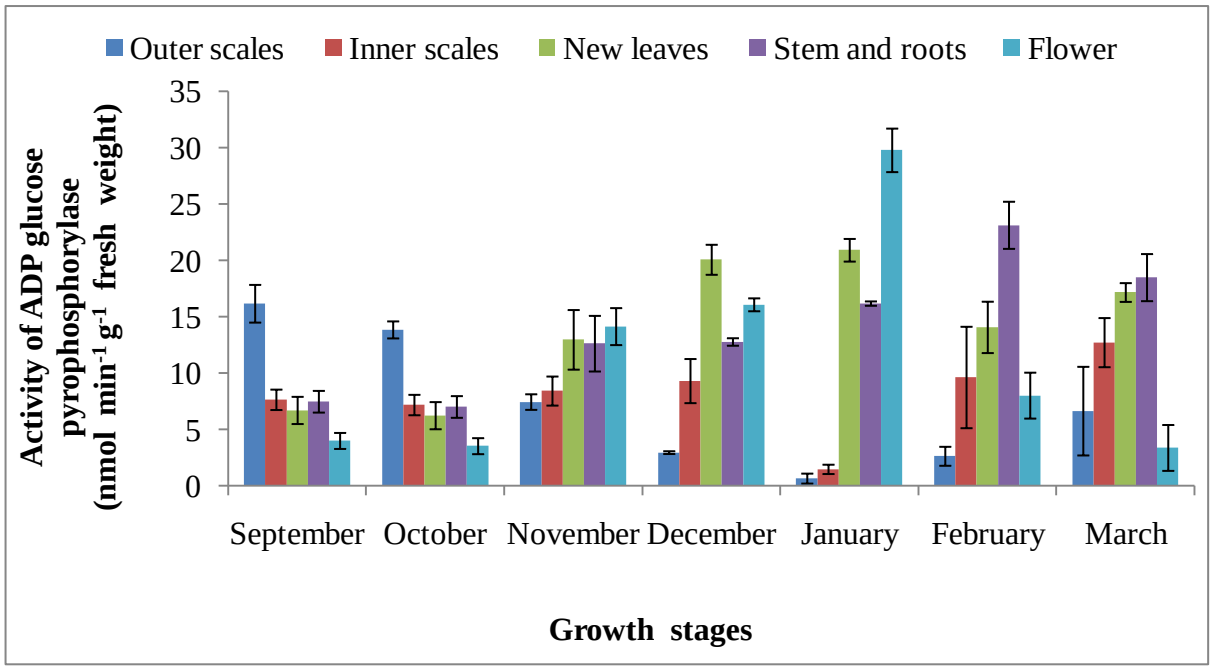


Fig 3.10: Variation in the activity of starch degrading enzymes during hyacinth bulb production in the 2009/2010 season: (a) α- amylase and (b) starch phosphorylase. Bars represent means ± SE of five replicates.

(a)



(b)

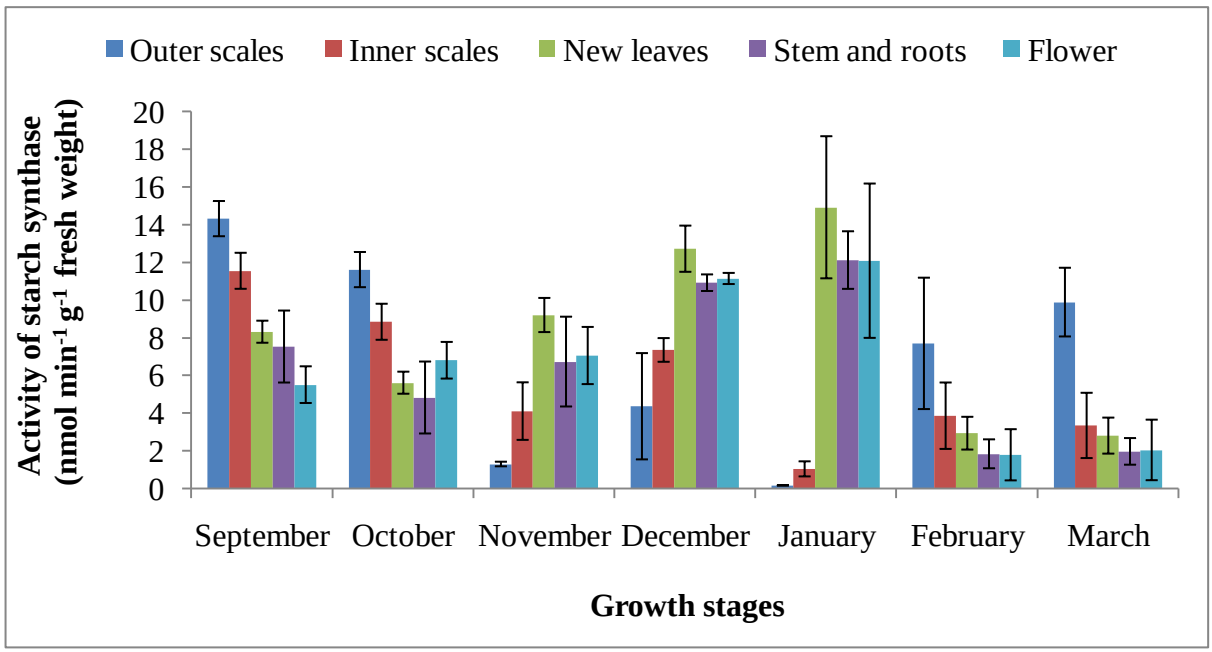


Fig 3.11: Distribution of activity of starch synthesizing enzymes during the growth of the common hyacinth in 2009/2010 planting season: (a) ADP glucose pyrophosphorylase and (b) starch synthase. Bars represent means ± SE of five replicates.

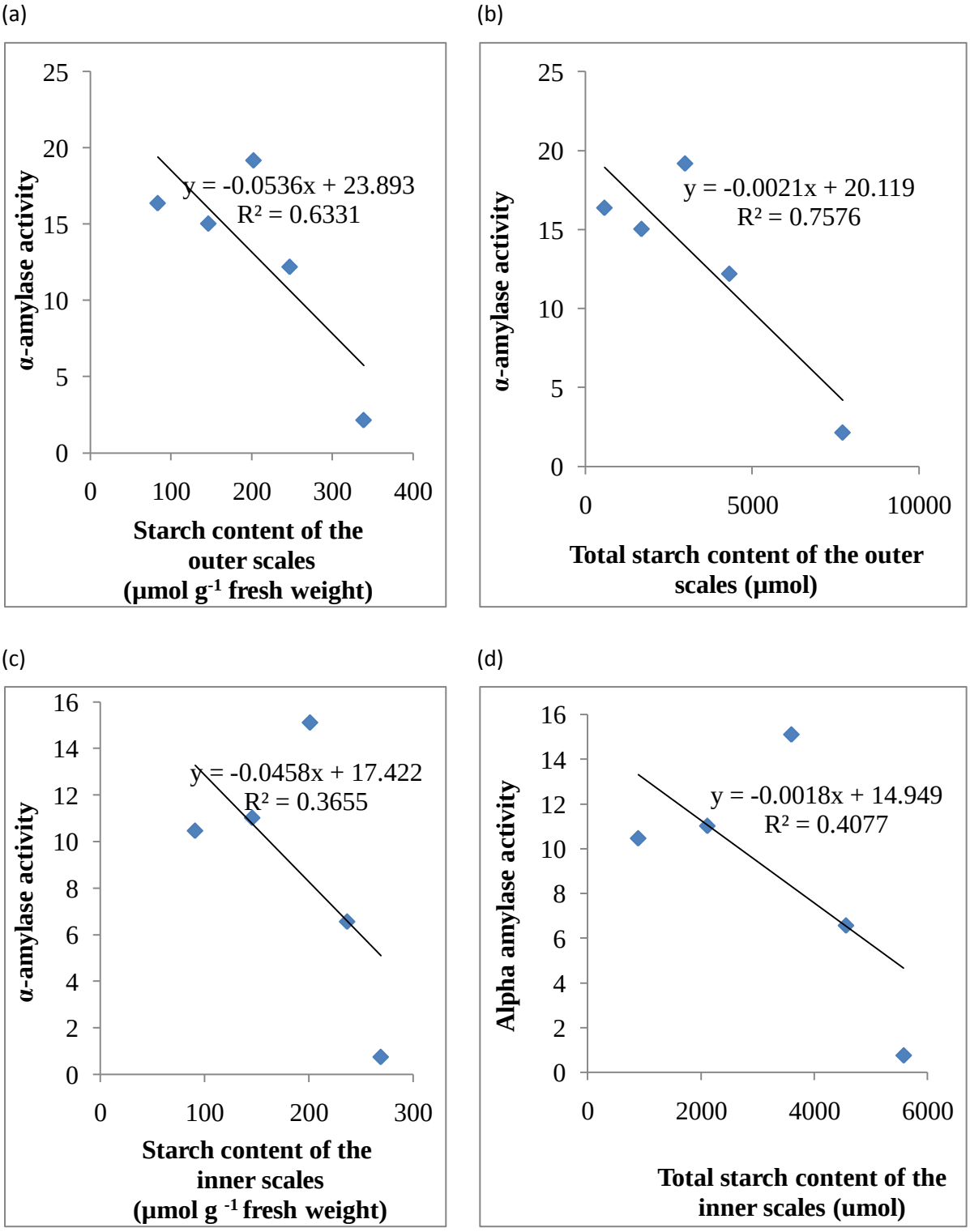


Fig 3.12: Relationship between α-amylase activity and starch degradation of the outer and inner scales of hyacinth. This figure demonstrates the correlation between the activity of α-amylase (expressed as µmol maltose min^{-1} g^{-1} fresh weight), and (a) outer scales starch content expressed as µmol g^{-1} fresh weight, (b) outer scales total starch content expressed as µmol, (c) inner scales starch content expressed as µmol g^{-1} fresh weight, and (d) inner scales total starch content expressed as µmol. The negative slope indicates that as the activity of the enzyme increases, starch degradation increases and hence the concentration of the carbohydrate decreases with time.

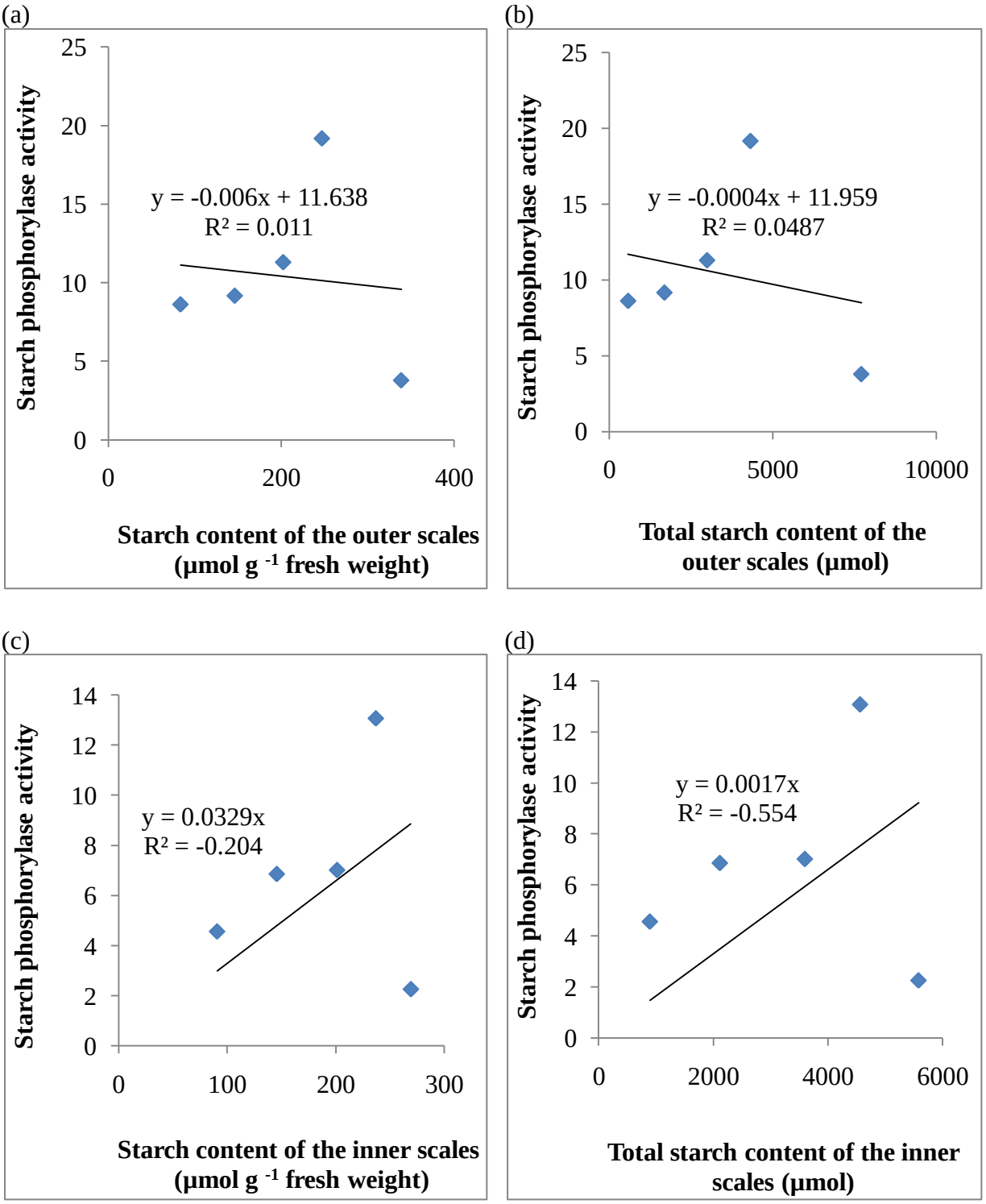


Fig 3.13: Relationship between starch phosphorylase activity and starch degradation of outer and inner scales of hyacinth. The figure shows the correlation between the activity of starch phosphorylase (expressed as mol P released min $^{-1}$ g^{-1} fresh weight), and (a) outer scales starch content expressed as µmol g^{-1} fresh weight, (b) outer scales total starch content expressed as µmol, (c) inner scales starch content expressed as µmol g^{-1} fresh weight, and (d) inner scales total starch content expressed as µmol. The negative gradients of (a) and (b) as well as the negative correlation of (c) and (d) imply that as the activity of the enzyme increases, starch concentration of the organ decreases with time.

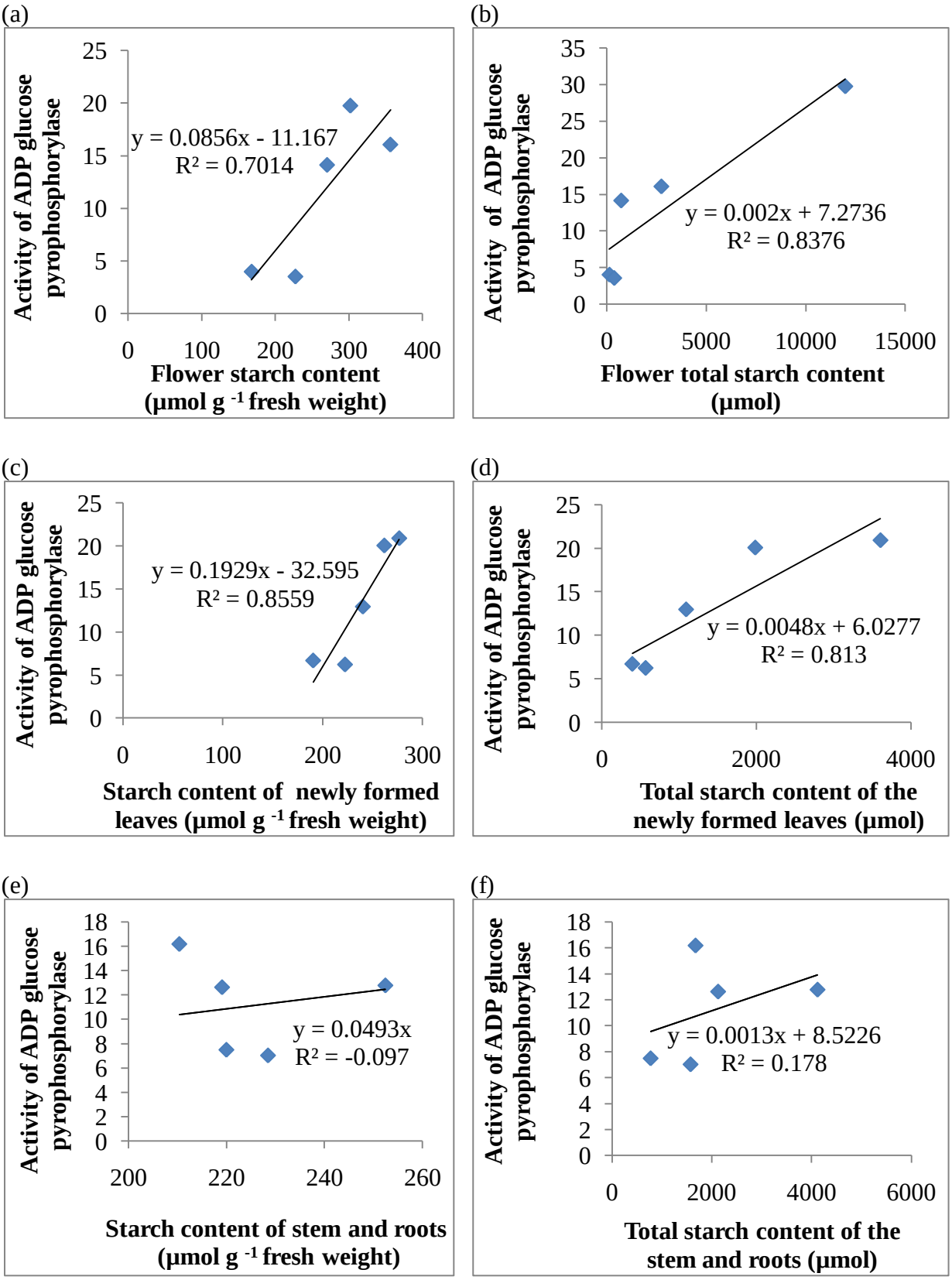


Fig 3.14: Relationship between the activity of ADP glucose pyrophosphorylase (expressed as nmol min^{-1} g^{-1} fresh weight), and starch accumulation of the flower (a, b), new leaves (c, d) and the stem and roots (e, f) of hyacinth. The positive slopes of the graphs suggest that the activity of this enzyme varies directly with starch accumulation.

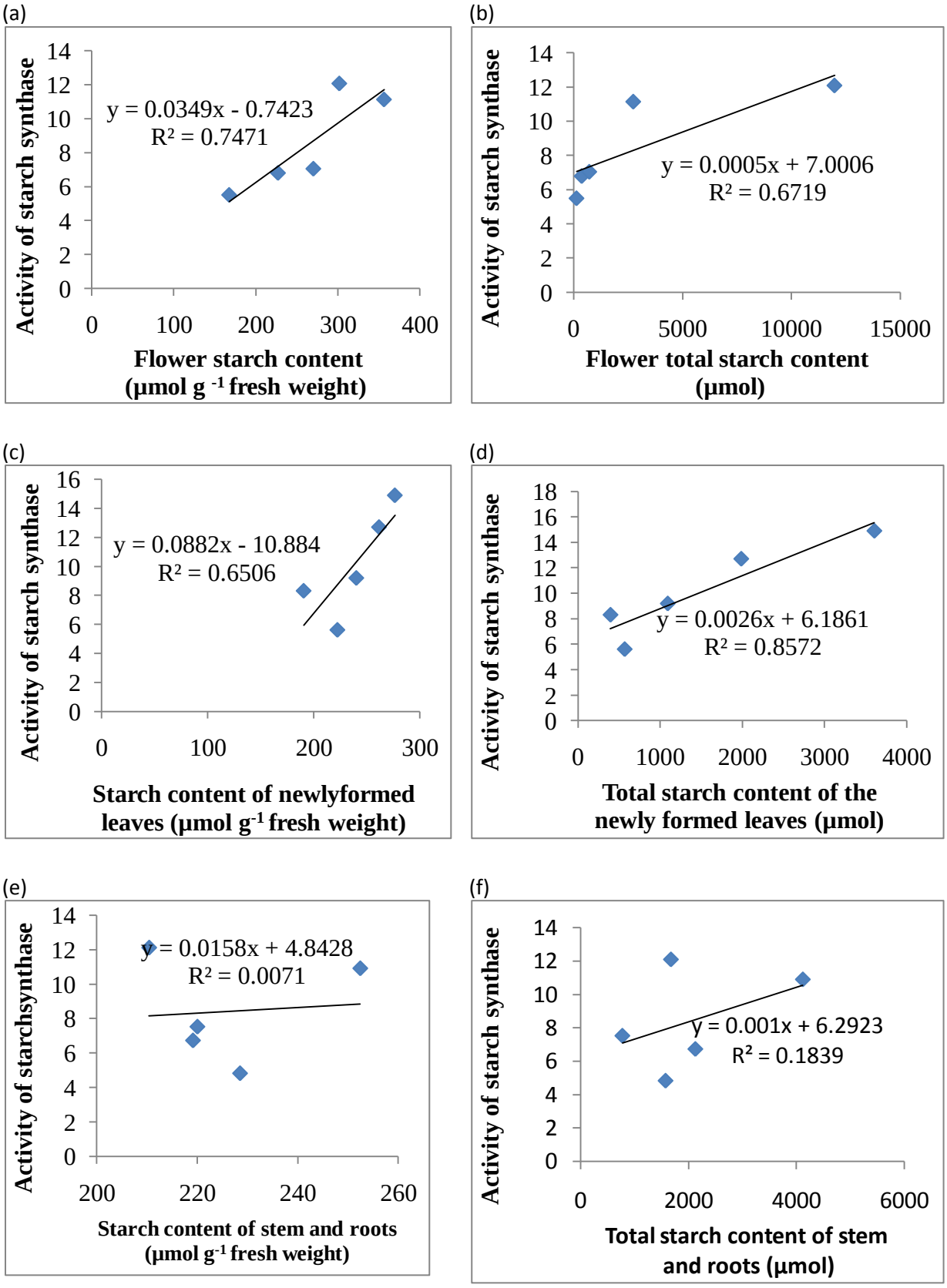


Fig 3.15: Relationship between the activity of starch synthase (expressed as nmol min^{-1} g^{-1} fresh weight), and starch accumulation of the newly formed organs of the common hyacinth. The figure shows that starch accumulation in these organs is proportional to the enzyme activity.

3.2.5 Carbohydrate metabolism during lily bulb production in the summer season of year 2008.

Hyacinth is a spring flowering bulb, and metabolism of carbohydrate of this bulb has already been investigated. This study also sought to research into carbohydrate metabolism in the lily, a summer-flowering bulb so as to have a fair knowledge about carbohydrate partitioning and metabolism in these two categories of flowering bulbous plants. Therefore between April 2008 and August 2008, lily bulbs were also planted and dissected into outer scales, inner scales, new leaves, stem and roots and flower at 0, 3, 6, 9 and 12 weeks after planting for starch, glucose, fructose and sucrose measurements in the same manner as that of hyacinth in the 2007/2008 planting season. Results on starch metabolism from this experiment were similar to that observed in the case of hyacinth. Biomass and starch of the scales were higher in the outer than the inner scales prior to planting; and the degradation of these parameters was higher in the former than the latter. Starch and biomass of both the outer and inner scales reduced from planting to nine weeks after planting and increased slightly from this point to twelve weeks after planting (Fig 3.16a and c; Fig 3.17b). Between 9-12 weeks after planting, there was no significant difference between fresh weight, dry weight and starch content of the outer scales and those of the inner scales (Table 3.3a; Fig 3.16a and c; Fig 3.17b). As was observed in the case of hyacinth, the degradation of starch and biomass of the scales of the lily led to the accumulation of these quantities in the newly formed organs (stem and roots, new leaves and flower) during the growth of the plant. However, whereas in the case of hyacinth, it was the flower (inflorescence) that recorded the highest accumulation of starch, biomass and growth (Fig 3.03b and d; Fig 3.04c and Fig 3.11a), it was rather the stem and roots that accumulated the highest amount of starch and biomass in the lily (Fig 3.16b and d; Fig 3.17c). At time zero, that is prior to planting of the bulb, starch content of the outer and inner scales were 283.25 and 169.85 µmol g^{-1} fresh weight, respectively, whilst glucose, fructose and sucrose levels for the outer scale were 22.3, 0.2 and 29.48, respectively. The amounts of these sugars recorded of the inner scales at the same time were 21.1, 0.4 and 22.46 µmol g^{-1} fresh weight, respectively (Table 3.3). In general, the levels of starch and glucose decreased after planting and the decrease was very sharp between 0 and 3 WAP, coinciding with the time of emergence of the shoot. This shows that, just like in the case of hyacinth, starch was the most dominant storage carbohydrate observed in the lily because values of the soluble sugars (glucose, fructose and sucrose) measured in the lily

bulb were low as compared to the amount of starch measured from the geophyte (Table 3.3). Moisture level of the scales generally increased from planting to six weeks after planting and gradually decreased from this point to twelve weeks after planting (Fig 3.17a). In the case of the new leaves and the stem plus the roots, moisture also increased from planting to three weeks after planting and then decreased gradually to twelve weeks after planting but the flower decreased in moisture from planting time to three weeks after planting, and between three to six weeks after planting, moisture increased again before it decreased to twelve weeks after planting. The outer scales recorded higher levels of all the three reducing sugars measured than the inner scales. The glucose level of the outer and inner scales as well as the stem and roots decreased from planting to three weeks after planting whilst fructose from these organs increased from planting to three weeks after planting indicating that glucose was mainly converted into fructose during sprouting since this time coincided with the emergence of the shoots from the soil (Fig 3.18). The glucose levels of the scales increased from three to six weeks after planting and then dropped to twelve weeks after planting. The level of fructose of the scales decreased sharply from three to six weeks after planting but from six to nine weeks after planting, the decrease in level of this carbohydrate was gradual up to twelve weeks after planting. Between six to twelve weeks after planting, levels of glucose and fructose of the outer and inner scales were the same. The sucrose level of the outer scales decreased, but gently from zero to three weeks after planting, then sharply from three to six weeks after planting and then gradually up to twelve weeks after planting but in the case of the inner scales, this sugar level decreased from zero to three weeks after planting, increased from this point to six weeks after planting and thereafter decreased up to twelve weeks after planting (Fig 3.18e). The flower gave the highest level of fructose and sucrose and the accumulation of these two sugars in the flower occurred between six and nine weeks after planting (Fig 3.18d and f).

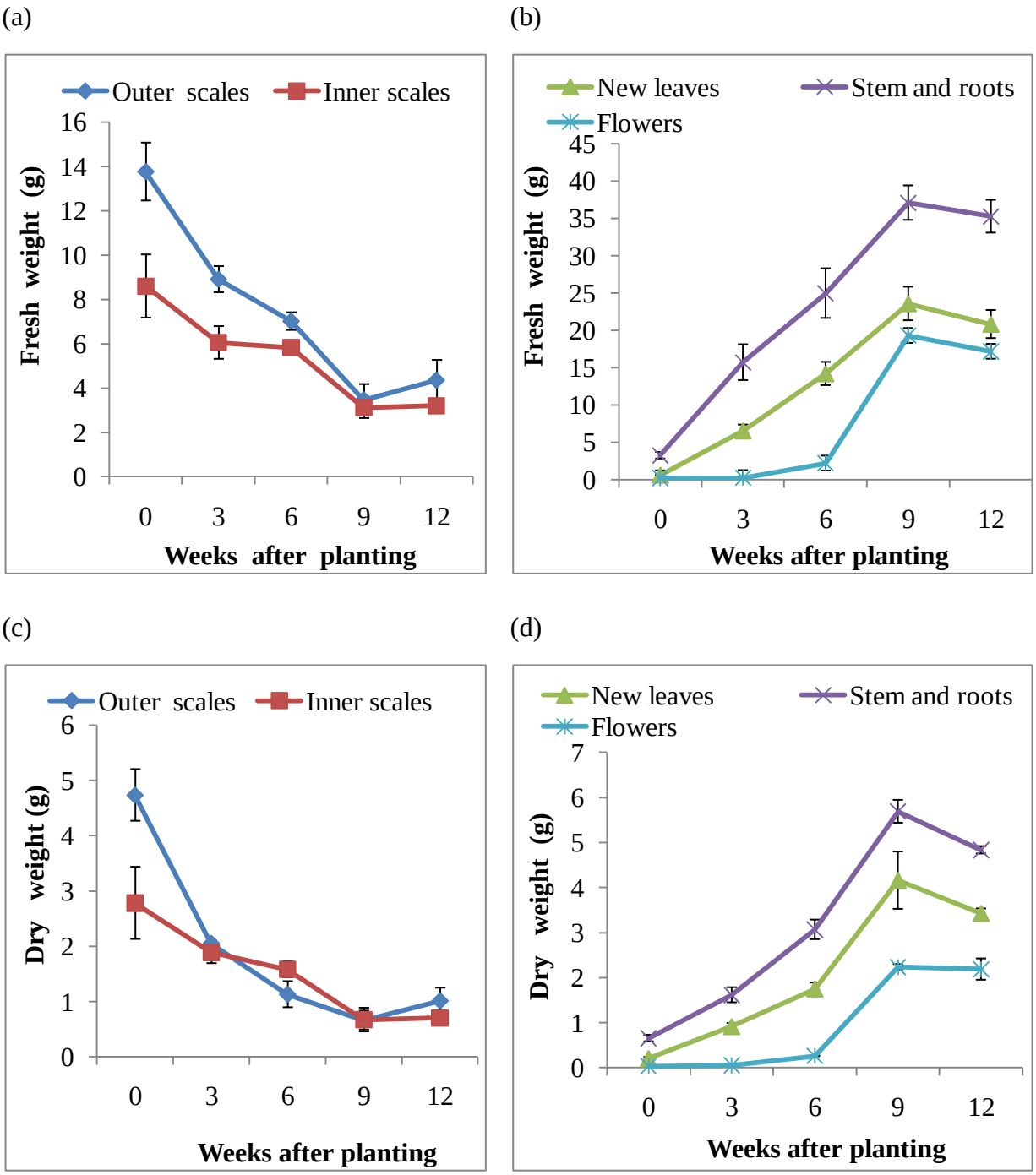
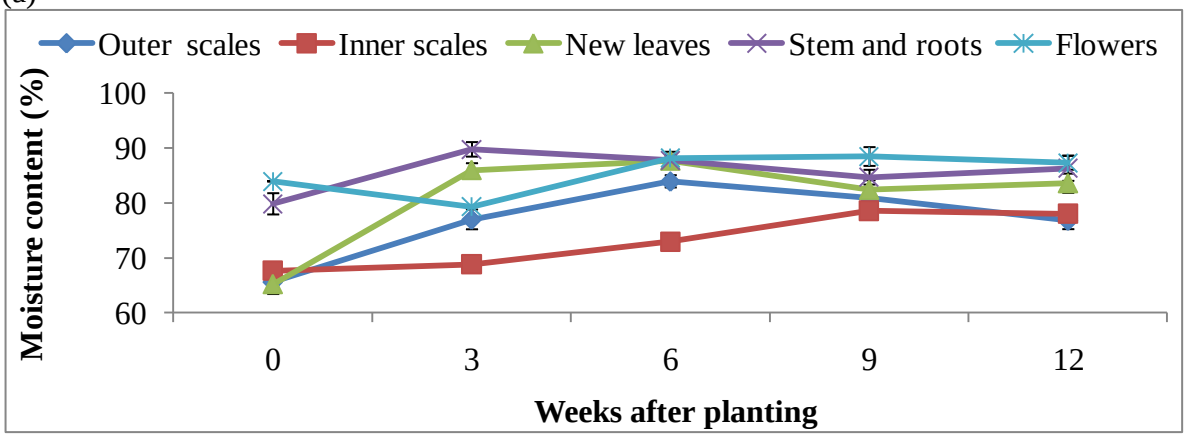
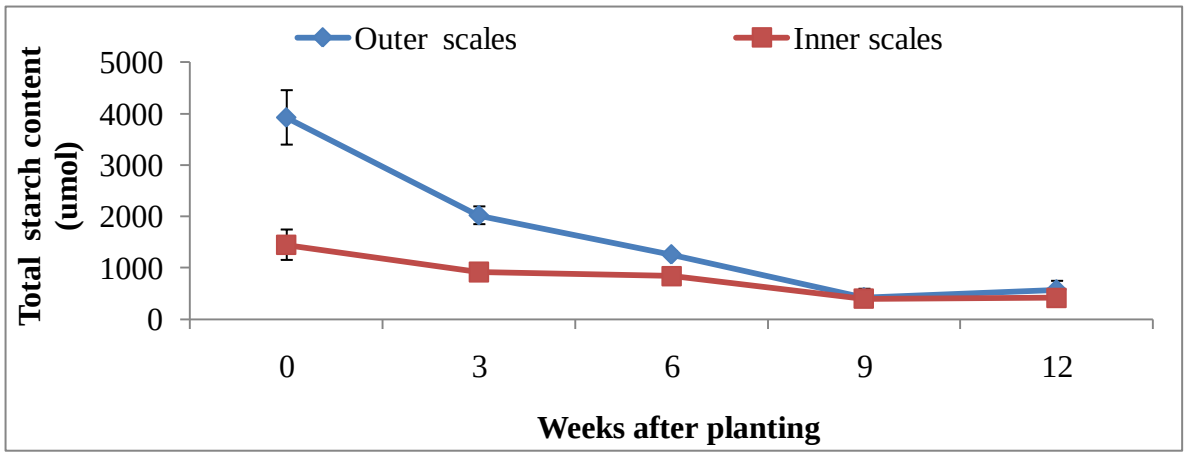


Fig 3.16: Variation in biomass of the lily organs. The figure shows the fresh weight of outer and inner scales (a), fresh weight of the newly formed leaves, stem and roots and flower (b), dry weight of the outer and inner scales (c), and dry weight of the new leaves, stem and roots, and the flower (d). Bars represent means ± SE of five replicates.

(a)



(b)



(c)

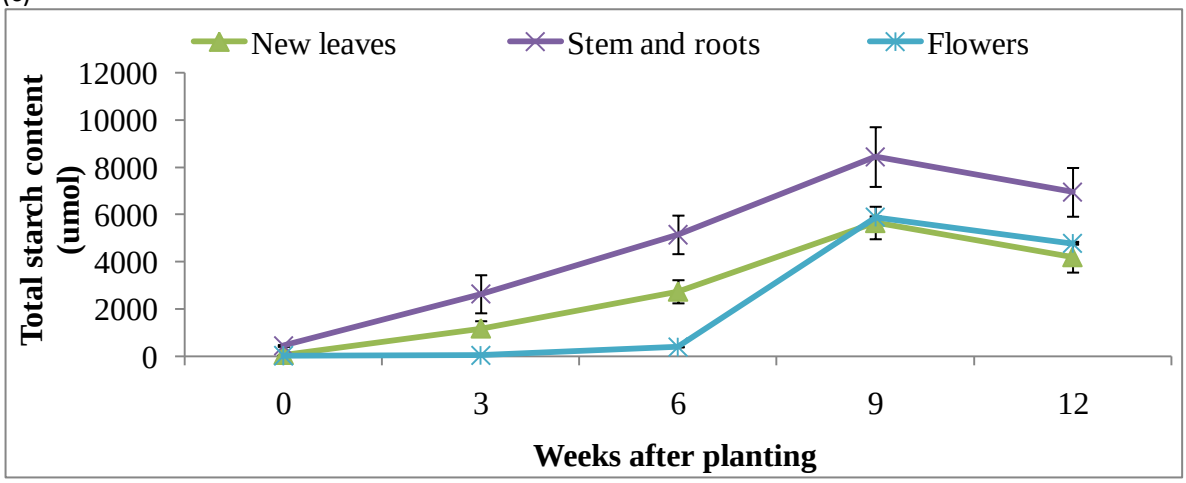


Fig 3.17: Variation in moisture and starch content of the lily organs. The figure shows the changes associated with (a) moisture content of the organs, (b) total starch content of the outer and inner scales, and (c) total starch concentration of the new leaves, stem and roots, and the flower (c). The bars represent mean values ± SE of five replicates.

Table 3.3 a: Changes in carbohydrates content of the lily organs: (a) Starch, (b) glucose. Values are in µmol g^{-1} fresh weight of the corresponding organs.

(a)

	Weeks after planting				
Organs	0	3	6	9	12
Outer scales	283.25 ± 18.01	203.06 ± 8.56	179.86 ± 9.32	120.23 ± 5.99	132.37 ± 6.12
Inner scales	169.85 ± 6.12	152.99 ± 10.09	143.79 ± 4.69	127.84 ± 1.97	130.63 ± 2.23
New leaves	79.26 ± 15.35	178.42 ± 6.87	192.59 ± 5.96	249.58 ± 9.61	201.56 ± 8.87
Stem and roots	137.33 ±8.42	176.69 ± 6.17	193.64 ± 8.53	221.74 ± 15.46	196.88 ± 13.46
Flower	80.67 ± 3.56	122.69 ± 30.94	176.48 ± 10.51	305.35 ± 10.56	277.52 ± 8.69

85

(b)

Outer scales	22.3 ± 0.23	1.2 ± 0.38	11.9 ± 0.25	5.6 ± 0.31	6.8 ± 0.41
Inner scales	21.1 ± 0.19	0.4 ± 0.15	10.5 ± 0.24	5.4 ± 0.18	6.1 ± 0.18
New leaves	12.7 ± 0.59	3.1 ± 1.12	9.2 ± 0.58	10.3 ± 0.92	9.9 ± 0.81
Stem and roots	13.2 ± 0.47	0.4 ± 0.12	6.6 ± 0.71	1.7 ± 1.11	1.6 ± 1.11
Flower	11.9 ± 1.94	0.5 ± 0.17	2.5 ± 0.39	12.6 ± 1.52	10.5 ± 1.32

Table 3.3b Changes in carbohydrates content of the lily organs: (c) fructose and (d) sucrose. Values are in µmol g^{-1} fresh weight of the corresponding organs.

(c)

	Weeks after planting				
Organs	0	3	6	9	12
Outer scales	0.2 ± 0.04	20.9 ± 0.73	0.2 ± 0.06	0.7 ± 0.16	0.7 ± 0.18
Inner scales	0.4 ± 0.19	9.8 ± 5.08	1.5 ± 0.08	0.6 ± 0.06	0.6 ± 0.08
New leaves	0.5 ± 0.17	17.8 ± 3.88	2.9 ± 0.58	4.1 ± 0.97	3.2 ± 0.87
Stem and roots	0.3 ± 0.08	20.6 ± 0.75	4.5 ± 0.62	3.9 ± 0.09	2.1 ± 0.07
Flower	2.8 ± 0.48	1.9 ± 0.41	6.7 ± 0.59	10.7 ± 0.63	8.5 ± 0.56

86

(d)

Outer scales	29.48 ± 0.26	21.06 ± 1.08	12.08 ± 0.27	6.23 ± 0.47	7.98 ± 0.57
Inner scales	22.46 ± 0.17	10.22 ± 5.21	11.94 ± 0.17	5.99 ± 0.19	6.87 ± 0.21
New leaves	13.28 ± 0.58	20.94 ± 2.99	12.16 ± 0.17	14.38 ± 1.15	12.06 ± 1.07
Stem and roots	13.49 ± 0.41	20.91 ± 6.41	11.03 ± 0.09	5.64 ± 1.09	4.45 ± 1.02
Flower	14.66 ± 1.53	2.34 ± 0.41	9.16 ± 0.79	23.28 ± 2.08	21.33 ± 2.01

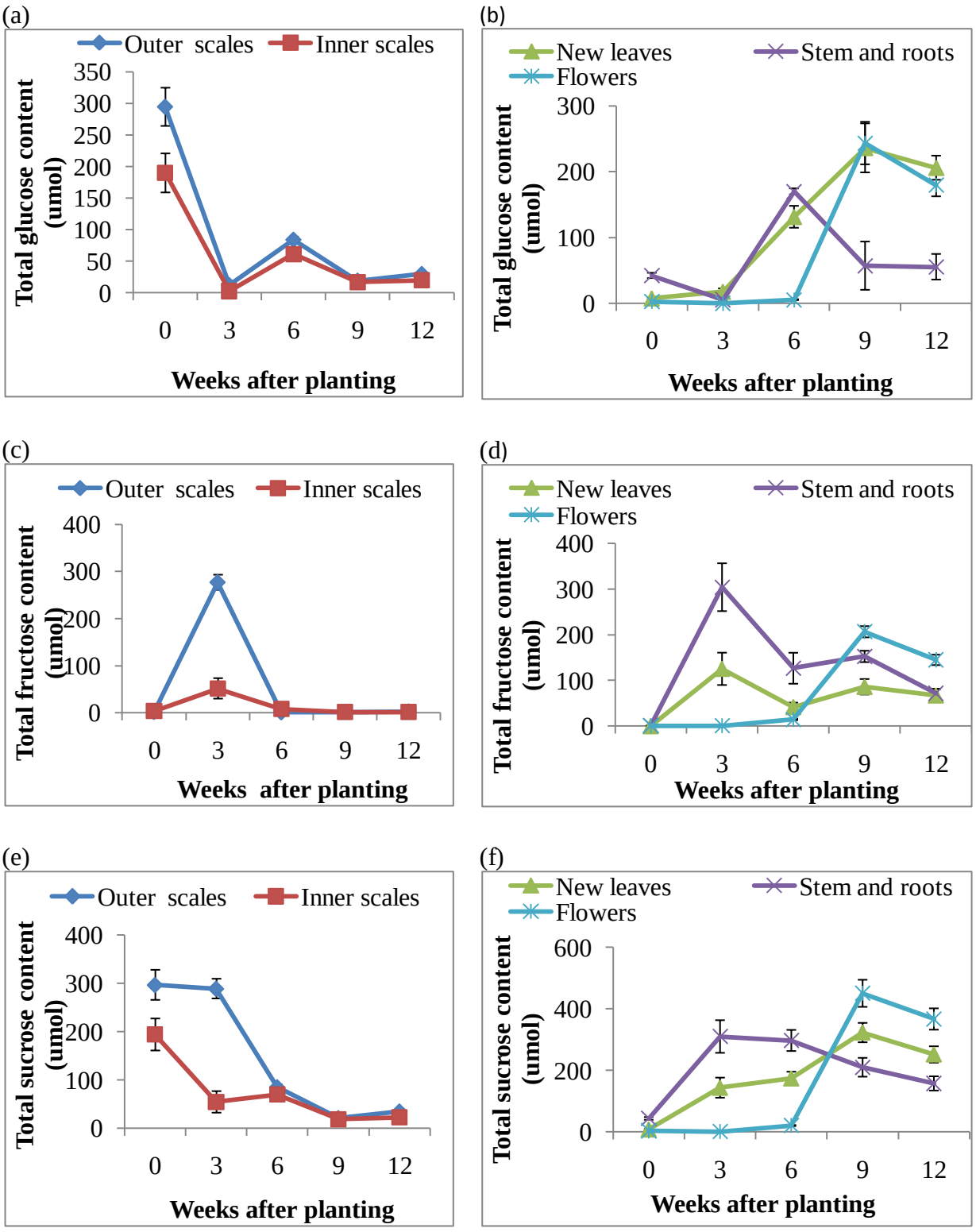


Fig 3.18: Variation in sugar content of the lily organs. The figure shows the changes in total glucose content of (a) the outer and inner scales, (b) the new leaves, stem and roots and flower; total fructose content of (c) the outer and inner scales (d) the new leaves, stem and roots and flower and total sucrose content of (e) the outer and inner scales (f) the new leaves, stem and roots and flower. Bars represent means ± SE of five replicates.

3.3 Discussion

The general reduction in starch and biomass of the scales of hyacinth and lily after planting of the bulbs is attributed to the occurrence of preformed shoots and roots. These newly developed structures made use of the energy stored in the bulbs for emergence and this is in accordance with the observation made by Theron and Jacobs (1996). According to these authors, reduction in reserve carbohydrate occurs in geophytes during the time of sprouting. It follows therefore that in horticultural bulb production, termination of dormancy or sprouting is connected with the breakdown of material accumulated in the storage tissues, in this case the bulb scales, and the utilisation of these materials is essential for the initial growth (Miller, 1992) and the development of the newly formed organs. Orthen (2001) also stated that starch was the major storage carbohydrates in bulbous plants and this carbohydrate served as the source of energy during sprouting. Carbohydrate measurements of the scales of both hyacinth and the lily also revealed that changes in dry weight (biomass) of the bulb scales followed closely that of starch. In the case of hyacinth, this was shown by the high correlation between the two parameters ($r = 0.694$, Table 3.1). Also, the outer scales recorded higher levels of starch and biomass than the inner scales prior to planting, but the rate of degradation of these parameters of the former was higher than that of the latter particularly during the first month after planting and in fact, starch content of the outer scales reduced to about 50 % of the initial value within one month after planting. The higher rate of starch degradation and depletion of the stored reserves of the outer scales as compared to the inner ones is also not uncommon in geophytes because Chen (1969) also made a similar observation in *Narcissus tazetta*. In the present study, the period of rapid reduction in starch also coincided with the time of sprouting of the bulbs. The high reductions in starch and biomass of the scales especially the outer ones during sprouting are also in agreement with the finding made by Du Toit *et al.* (2004). They reported that in *Lachenalia*, the total dry weight of the bulb and starch content decreased by 80 and 90 %, respectively, at six weeks after planting that is during sprouting of the bulb. Lambrechts *et al*. (1994) also observed that the dry weight of the mother bulb scales of tulip decreased to half of the original value at the time of sprouting. A similar reduction in weight and starch content was observed by Ohyama *et al.* (1998). The observation made in this work concerning the differences in starch and biomass content of the outer and inner scales and their rate of depletion

with time is in conformity with Miller's (1992) observation that starch content of a plant varies from time to time and from one organ or tissue to another and also the finding made by Miller and Langhans (1989) that differences occurred in the rate and capacity of depletion in bulb scales. In 2006/2007, L5, L6 and L7 accumulated starch from two to three months after planting and this also reflected in their dry weight measurements. Similarly, there was a general rise in starch and dry weight of all scales during the 2006/2007 season (Fig 3.02a) from five to seven months after planting. The accumulation of these quantities was as a result of assimilates received from current photosynthesis since photosynthetic leaves had already formed at this time of growth. It is also important to note that in 2006/2007, scales L1, L2, L3 and L4 behaved similarly whilst L5, L6 and L7 also had a similar metabolic function with regard to their content of starch and biomass as well as the rate of disappearance of these parameters. Thus L1-L4 and L5-L7 were classified as outer and inner scales, respectively. The high values of starch compared with the generally low values of the soluble sugars of the hyacinth scales (Table 3.2) and the gradual reduction and disappearance of sugars from four to seven months after planting as well as the accumulation of starch in the scales from five to seven month after planting during the 2006/2007 season imply that starch was the major storage carbohydrate in hyacinth. Vishnevetsky *et al*. (2000) also worked on *Nerine sarniensis* cv Salmon and reported that starch was the dominant storage carbohydrate in that bulb. The reduction in starch and biomass of the scales of hyacinth in 2007/2008, and that during the summer of year 2008 by the lily was accompanied by an increase in these parameters of the new leaves, stem and roots and flower. The accumulation in biomass and starch of these bulbs also reflected in the general growth of these newly developed organs. This is consistent with the observation made by Theron and Jacobs (1996) that reserves stored in bulbs are used for the development of newly formed organs and once the leaves mature to become the photosynthate source, resources may be stored in the old and new leaf bases. Similarly, Wassink (1965) concluded that in bulbous plants, the aerial parts develop mainly at the expense of the mother bulb. The depletion of scales in biomass and starch and the concomitant accumulation of these resources in the newly developed organs especially during the early stages of growth before photosynthetic leaves were developed also means that the resources were being exported from the scales into these newly formed organs. In hyacinth, it was the flower that accumulated the greatest amount of biomass and starch whilst in the case of the lily, it was the stem and roots that accumulated the highest

amount of biomass and starch. In bulbous plants, therefore, one cannot rule out the importance of this export of materials or nutrients (reserve carbohydrates) from the scales to the newly formed organs. The greatest contribution of this export of reserves is from the outer scales since its rate of carbohydrate degradation was faster than that of the inner scales. There is thus a connection between the rate of depletion of nutrients from the scales and their accumulation in the newly formed organs. It is therefore not surprising that when hyacinth or the lily (but particularly hyacinth) was subjected to bulb herbivory (scale removal), flower/inflorescence development was inhibited (See Chapter 4 of this thesis where the influence of simulated bulb herbivory was discussed). It is very important to mention that as the intensity of peeling of the bulb increases, inflorescence formation (inflorescence height, length, diameter and number of florets) decreased both in hyacinth and the lily, emphasizing that peeling of the bulb took away some reserve carbohydrates needed for growth and development of the bulb especially in the case of inflorescence development. This also explains why 100 % scale removal (peeling away both the outer and inner scales) was more detrimental to flowering and productivity of the bulbous plant than 50 % (only the outer scales being peeled away). Die *et al. (*1970) also observed that the flower–bearing part of the shoot of bulbous plant was an important sink for assimilates.

The metabolism of sugars in the lily was remarkable: from 0 to 3 weeks after planting (sprouting), both glucose and sucrose of the outer and inner scales were metabolised (Fig 3.18), indicating that metabolism of glucose, sucrose and starch provided energy that fuelled the emergence of shoots and roots since that period coincided with sprouting of this bulb. However, fructose accumulated in the scales at this period and this also implies that during sprouting of the lily bulb, there was an inter-conversion of starch or glucose to fructose. The flower accumulated the highest amount of the soluble sugars mainly glucose and sucrose. The metabolism of these two sugars as well as starch in the scales of the lily was thus responsible, at least in part, for flower development in the lily.

Studies involving planting of hyacinth bulbs in the soil and glass tubes showed that planting of this bulb in the glass tube during the 20-day period resulted in lower reductions in biomass and starch as compared to planting in the soil. The high reduction in biomass and starch of bulbs planted in the soil was due to early development of roots

and shoots that characterised planting in the soil. It has already been established that in bulbs like many other geophytes, the newly formed organs (aerial parts) utilised the energy stored in the bulb (underground part) for emergence and growth. During the experimental period of 2008/2009, root developed as early as 4 days after planting from bulbs that were planted in the soil so that by 20 days after planting, leaves had already started developing and emerging from bulbs planted in the soil whereas the emergence and development of these organs was delayed in the glass tubes. This observation is in agreement with that made by Nowak *et al.* (1974). According to them, when rooted and unrooted bulbs of hyacinth were exposed to a period of low temperature, the amount of carbohydrate initially present in the scales decreased, though the decrease was greater in rooted than the unrooted bulbs. Also the activities of hydrolytic enzymes were significantly higher in rooted than unrooted bulbs. In general, therefore, since bulbs planted in glass tubes recorded lower percentage reductions in biomass and starch than those planted in the soil, it follows that planting in the glass tubes offered the opportunity to minimise the high reductions in starch and biomass that characterised the early stages of the bulbs when they are planted in the soil.

The distribution of the hydrolytic enzymes closely followed the pattern exhibited by starch degradation in the scales of hyacinth. The activities of both α-amylase and starch phosphorylase were generally higher in the outer scales than in the inner scales confirming the earlier observation that starch is degraded to a higher extent in the outer than the inner scales. This observation is also confirmed by the higher correlation coefficient ($r^2 = 0.6331$ or $r^2 = 0.7576$, Fig 3.12a and b) between the starch content of the outer scales and α-amylase activity compared with the lower correlation between the starch content of the inner scales and the enzyme activity ($r^2 = 0.3655$ or $r^2 = 0.4077$, Fig 3.12). This is in agreement with the statement made by Banaski *et al*. (1980) that α-amylase activity and size of starch grains increased within a bulb from the inner to the outer scales. Komiyama *et al.* (1997), however, reported that amylase activity in the storage organs of flowering bulbs generally increased slightly during cold periods but the increase became dramatic during shoot elongation. In the present study, the activity of α-amylase increased from planting (September) to November (three months after planting) and this is the period where starch degradation was at its peak in hyacinth. There was a reduction in activity of this enzyme at three months after planting because during this period starch was degraded to a lower extent in the scales of hyacinth. Starch

phosphorylase activity increased from September to October (one month after planting) coinciding with the period in which sprouting of the bulb of hyacinth normally occurs. In hyacinth bulbs, sprouting usually occurs at a period around one month after planting when the environmental conditions are favourable for growth. This observation is thus in accordance with the finding made by Akazawa and Hara–Nishimura (1985). According to these authors, alpha amylase activity is known to increase during germination of seeds or sprouting in most geophytes. The correlation studies (Fig 3.12-3.13) also revealed that α-amylase better related with starch degradation in hyacinth than did starch phosphorylase (Fig 3.12-3.13). Also the activity of ADP glucose pyrophosphorylase of the scales particularly that of the outer scales decreased from September to January and rose to March whilst the activity of the same enzyme generally increased from planting (September) to January (four months after planting) for the newly formed organs (stem and roots, new leaves and flower), indicating the accumulation of starch in these organs. The trend exhibited by starch synthase was similar to that of ADP glucose pyrophosphorylase but these two enzymes correlated better with starch accumulation of the flower and the newly formed leaves than they did the stem and roots (Fig 3.14 and 3.15).

CHAPTER 4
HERBIVORY AND DROUGHT STRESS

4.1 Introduction

In flowering bulbs, the underground portion of the plant serves either as sink organ or source organ. Before new leaves are fully developed to provide photosynthates, growth in bulbs and other geophytes depends on reserves deposited and stored in the bulb and leaf bases during the preceding season. These reserves are used for the development of new leaves and roots. However, once the leaves are fully developed to become the photosynthate source, resources are stored in the old and new leaf bases. The inflorescence then becomes the major sink when elongation of the flower stalk begins (Theron and Jacobs, 1996). It is known that in bulb production, damage to the foliage or the underground portion (this may occur as a result of field physiological disorders, such as outbreak of diseases or pests, or when there is hail or frost during unfavourable weather) may not only affect the source-sink relationship but also reduce growth and yield of these plants. According to Rees (1972), physiological disorders may cause either a complete failure of flowering or contribute in one way or the other to reduce growth. However, some of the disorders have no known identified causal organisms but they are attributable to unfavourable environmental conditions. The causes, symptoms and control measures of most disorders of bulbous plants have already been discussed under Chapter 1, but it is of importance to stress that in hyacinth the disease called 'fire', caused by *Botrytis hyacinthi*, or bacterial soft rot disease caused by *Bacterium carotovorum*, or the yellow spots disease by *Xanthomonas hyacinthi* may spread to destroy the foliage, the inflorescence and the bulbs, respectively. Similarly field-grown lily bulbs may suffer from *Botrytis* and root-rot (Dimock and Tammen, 1967). Brewster (1994) also enumerated a number of factors that may limit the yield or affect the performance of plants as follows: the quality of light absorbed by the leaves while dry matter is being produced; the efficiency with which the absorbed light is converted by photosynthesis into sucrose; the proportion of photosynthetic output transferred to plant parts; the conversion coefficient between photosynthetic sucrose and the biochemical constituents of the harvested material and the weight losses due to respiration and decay after the above photosynthetic and biosynthetic processes have occurred. Any damage to the flowering bulb during the growth of the plant or prior to planting the bulb, as a result of physiological disorder or unfavourable environmental conditions may negatively affect any of the above mentioned processes and this will result in poor growth and development.

In general, it is known that herbivory plays an important role in the organization of plant communities as well as the evolution of their constituents species. Herbivory influences plant distribution and structure of the community (Haper, 1969). In view of the above-mentioned importance of herbivory, the phenomenon is also known to impact negatively on plant growth and development. For instance, Rockwood and Lobstein (1994) reported that in plants, herbivory may decrease growth and reproduction, increase probability of mortality or reduce the leaf area available for photosynthesis. Marquis *et al*. (1997) also observed that when a plant loses its tissues or organs as a result of a stress, they generally use their stored compounds to replace the tissues lost by browsing of photosynthetic area and for future support of biosynthesis for growth or other functions. McNaughton (1983) and Rosenthal and Kotanen (1994) stated that factors such as timing, type and extent of herbivory as well as the availability of resources in the environment to support growth may influence plants' response to herbivory. Similarly, Lennartsson *et al*. (1997) reported that the grazing or browsing history of the plant may also affect plants' response to herbivory. Plants generally store more resources to support growth and reproduction when they are subjected to stresses (Chapin *et al*., 1990; Dafni *et al*., 1981). Therefore in arid environments, since plants are exposed to drought, they show adaptations that enable them to frequently accumulate large nutrient stores to allow them to respond quickly to a period of water availability (Boeken, 1990). Dure (1993) also stated that plants that are exposed to moisture-deficit environments have reduced growth rates and in some cases, some proteins may play a role in protection of other proteins or membranes, so as to preserve cells structural integrity (Close, 1997), or they may also act as regulators of cell osmotic potential (Nylander *et al*., 2001) and supplement the protection afforded by sucrose accumulation (Scott, 2000). Similarly, bulbs grown on fields having inadequate irrigation practices especially during hot summer seasons may suffer drought stress and this could result in dehydration of plant parts at some point during their life cycle and thereby leading to the production of poor quality materials or a reduction in yield at harvest. However, in flowering bulbs, since the bulb is a storage site not only for food reserves but also water, it would be anticipated that the bulb may be able to tolerate, at least, some degree of water stress. The response of plants to biotic or abiotic stresses could be best studied and damages quantified when the plants are subjected to these effects under controlled environments. Therefore, bulbs of the common hyacinth (*Hyacinthus orientalis*) and lily (*Lilium longiflorum*) were planted, and subjected to

simulated herbivory (below- and above-ground herbivory) and drought stress during the 2006/2007 and 2008/2009 planting seasons, with a view to investigating into the effects of these treatments on growth, development and yield of these plants. Understanding the physiological mechanisms of these bulbs to cope with herbivory or water stress in relation to their growth, flower and bulb production would not only allow growers to produce good quality bulbs but will also allow scientists particularly breeders to develop efficient strategies to screen available germplasm or lines of these bulbs to identify genotypes that could be resistant to, or escape damages caused by herbivores, diseases or drought.

4.2 Results

4.2.1 Influence of scale removal on growth and development

4.2.1.1 Hyacinth

Two sets of experiments were conducted on hyacinth with a view to investigating the effects of bulb scale removal on growth and productivity of this flower bulb. The first of these two sets of experiments was carried out between November 2006 and June 2007. In this experiment, bulbs of the common hyacinth were subjected to 50 % scale removal (i.e. half of the scales tissues of each bulb were removed by peeling) before planting (BP) and at two and three months after planting (MAP). Similar bulbs were planted without the scale removal treatment and these served as the control. Variations in the greenhouse temperature, relative humidity and light intensity during this season were as presented in Figure 4.03. The second experiment on response of hyacinth to scale removal was conducted during the planting season of October 2008 to May 2009. In this experiment, bulbs of hyacinth were subjected to either a complete (100 %) scale removal or partial (50 %) peeling before planting or at 2 weeks after planting (WAP), and plants from these regimes were compared to those produced from the unpeeled control. Figure 4.10 summarises the changes in weather parameters during the experimental period of the second experiment.

Results during the 2006/2007 planting seasons revealed that hyacinth plants produced from bulbs whose scales were removed (peeled) prior to planting took longer to emerge from the soil than those from the unpeeled control (Fig 4.02a). That is, whereas the unpeeled bulbs sprouted in 51 days on average after planting, peeled bulbs sprouted 59 days on average after planting. Peeling also reduced vegetative growth (Fig 4.01a, b and c) because all hyacinth plants produced from peeled bulbs had lower leaf widths and lengths and this also reflected in their reduced leaf area values. From 13 to 21 weeks after planting, that is during data collection period, of the 2006/2007 planting season, maximum and minimum temperature in the greenhouse varied from 16.06 to 30.93 and 1.0 to 7.0^{o}C, respectively, whilst the maximum and minimum relative humidity also reduced from 61.99 to 44.66 % and 31.55 to 19.01 % respectively. At the same time, light intensity ranged from 121.4 to 714.9 x 100 lux (Fig 4.03). At 21 weeks after planting, total leaf area value of plants produced from the unpeeled control was 512.4 cm^2, whilst those from 50 % peeled bulbs prior to planting regime had a value of 416.9 cm^2, representing 81.36 % of that of the control in 2006/2007. The results in 2006/2007

also showed that plants produced from bulbs that were 50 % peeled prior to planting produced higher leaf growth than those whose bulb scales were peeled two and three months after planting. Scales removal also gave rise to plants with reduced inflorescence height (Fig 4.02b) because plants produced from bulbs of all peeling regimes recorded significantly lower values of inflorescence height than those from the unpeeled control. Plants from unpeeled bulbs recorded a mean of 15.5 cm of inflorescence height whilst those from bulbs peeled prior to planting had an average of 8.6 cm, implying that peeling resulted in a reduction of 44.5 % in terms of inflorescence growth. Additionally, a comparison of all the three peeling regimes revealed that plants from bulbs whose scales were removed prior to planting had lower inflorescence height than those produced from bulbs that were peeled either two or three months after planting. However, plants from bulbs peeled two or three months after planting produced similar values of inflorescence heights. Bulb fresh weight at harvest (Fig 4.02c) gave a distribution pattern that closely mimicked the one depicted by leaf growth.

Results of below-ground herbivory of hyacinth during the 2008/2009 planting seasons were similar to those of 2006/2007. Plants produced from all the peeled bulbs had reduced leaf growth (Fig 4.04 and 4.05) as compared to those from the unpeeled control. In this season, data was collected from 12 to 24 weeks after planting during which time the maximum and minimum temperatures ranged from 16 to 32^{o}C and -4 to 9.9^{o}C, and maximum and minimum relative humidity varied from 63.9 to 73 % and 23 to 47 %, respectively. Also, light intensity increased from 24 to 650 x 100 lux (Fig 4.10). Similarly as indicated above for 2006/2007, in 2008/2009, at 21 WAP (Fig 4.04), plants from the control recorded a leaf area value of 401.71 cm^2 whilst those from the 50 % peeling prior to planting regime had a total leaf area of 132.19 cm^2, representing 33 % of those of the control. The 100 % scales removal regimes produced plants with a greater reduction in leaf growth parameters (Fig 4.05) than their counterparts from the 50 % scales removal treatments. Also during the 2008/2009 season, plants produced from either the 50 or 100 % scales removal at two weeks after planting had higher leaf growth values than their counterparts from bulbs whose scales were removed before planting. Peeled bulbs recorded lower chlorophyll values than those from the unpeeled control (Fig 4.06). The chlorophyll concentration of plants produced as a result of scales removal in 2008/2009 produced a distribution that mimicked the pattern exhibited by

the leaf growth during that season. That is, plants produced from bulbs peeled at two weeks after planting recorded higher chlorophyll values than their corresponding counterparts whose scales were peeled before planting.

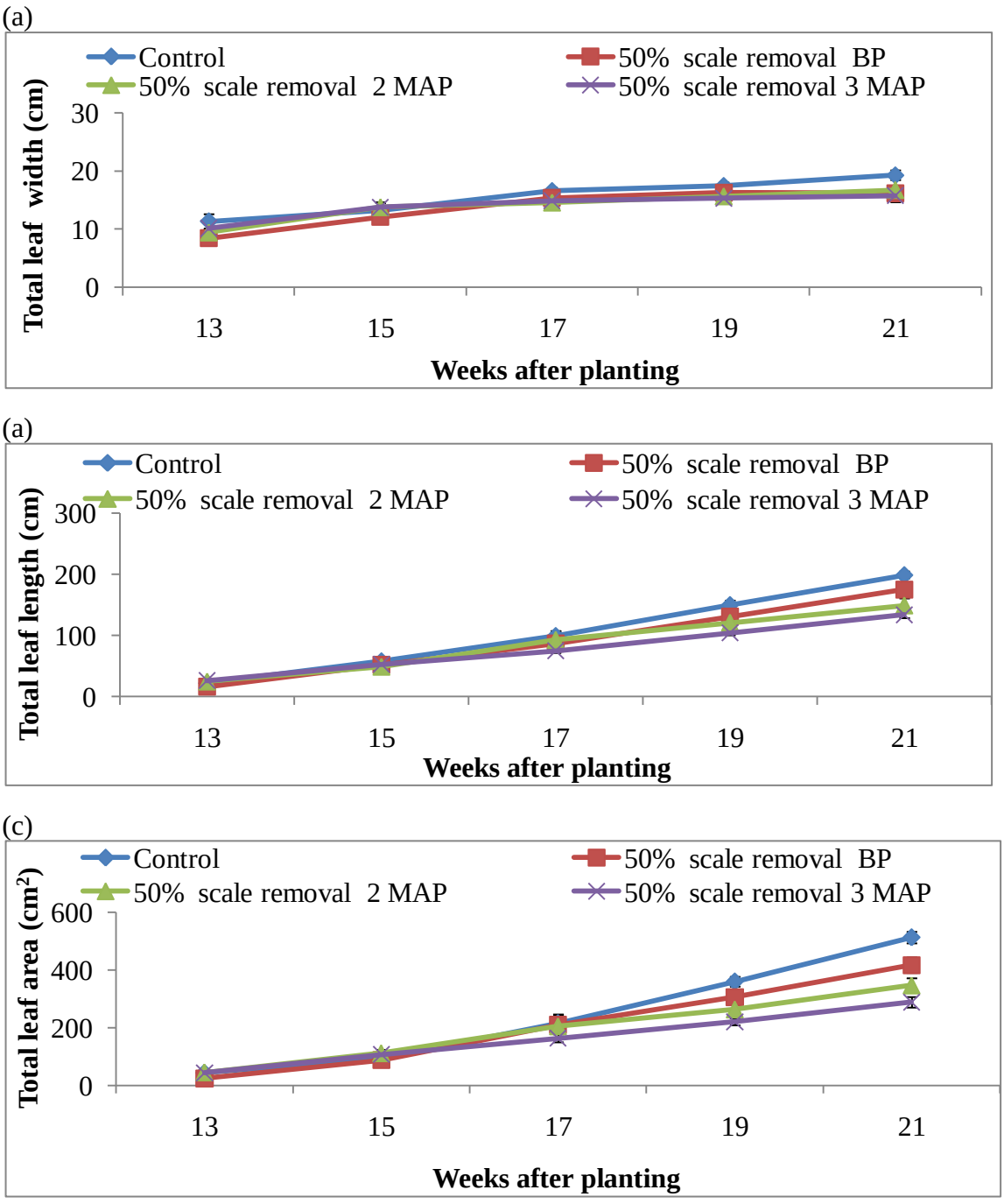
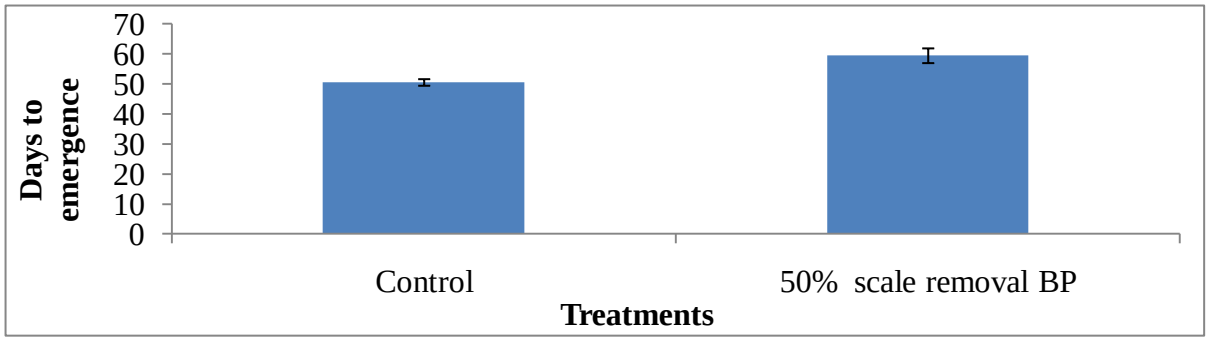


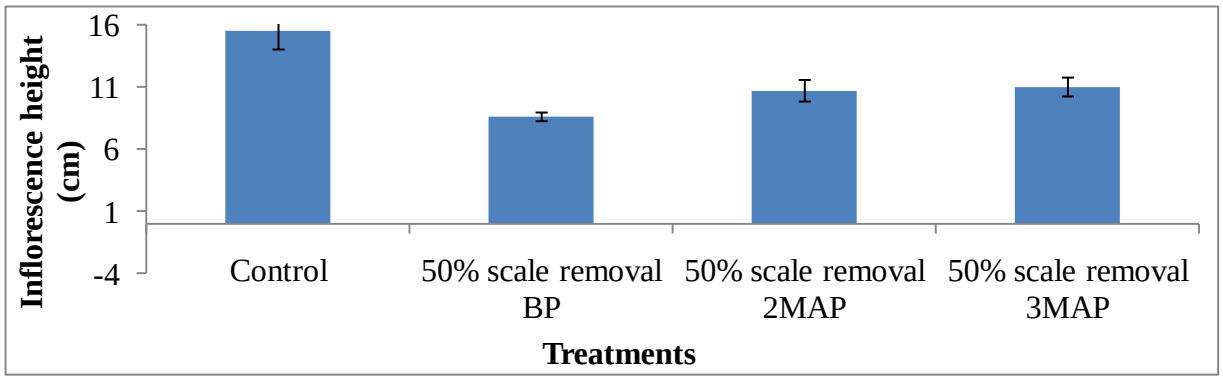
Fig 4.01: Influence of 50 % (partial) scale removal on hyacinth leaf growth: (a) total leaf width, (b) total leaf length and (c) total leaf area during the 2006/2007 planting season. Bars represent means ± SE of four replicates.

Plants produced from peeled bulbs prior to planting emerged at 51 days after planting, whilst those from the unpeeled control took on average 59 days to emerge from soil. Plants whose bulbs scales were peeled at 2 or 3 MAP emerged at the same time as the control (i.e. before peeling at 2 or 3 MAP, these plants were just like the unpeeled control).

(a)



(b)



(c)

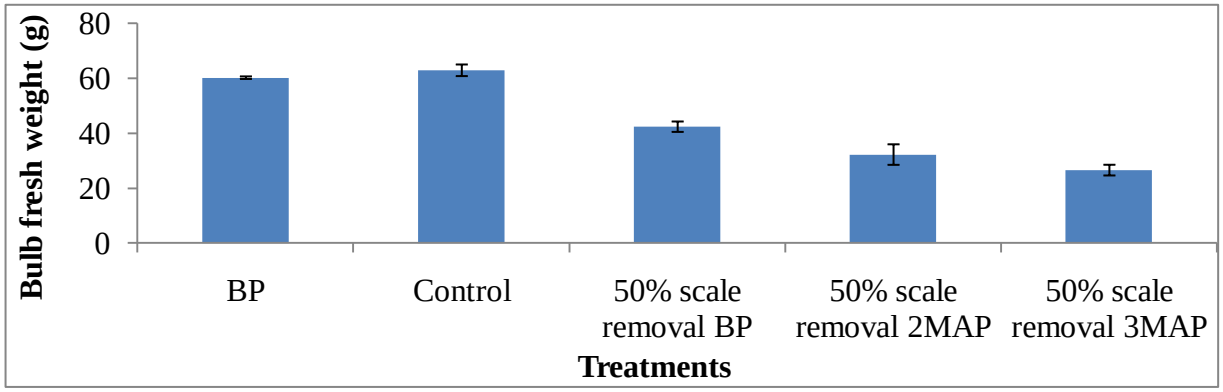
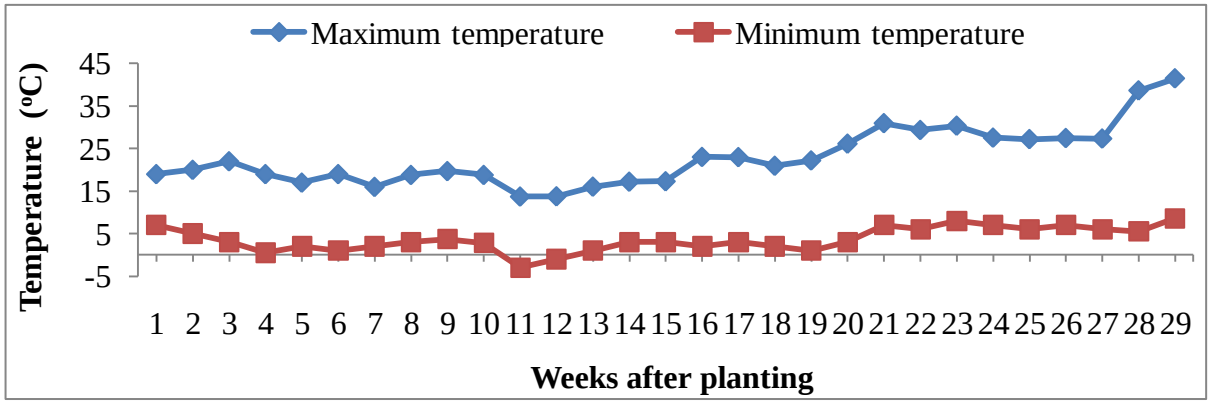
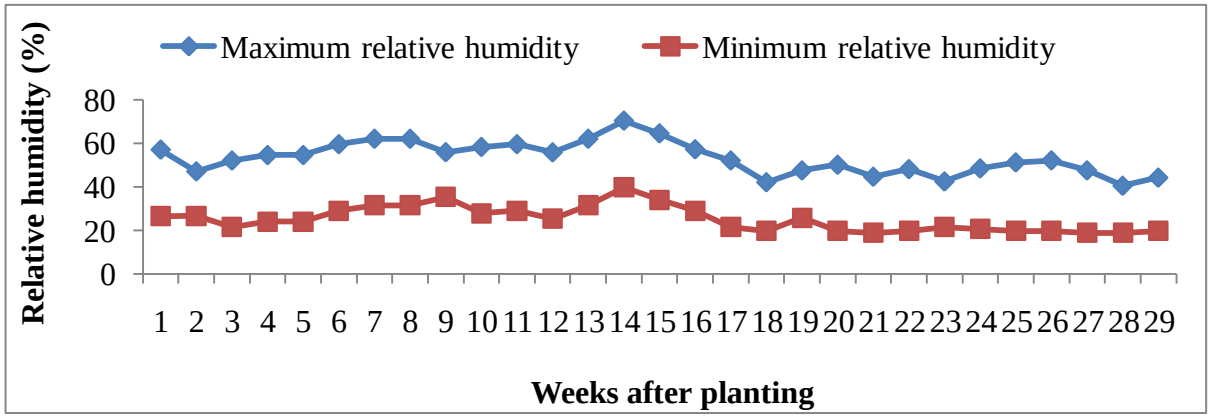


Fig 4.02: Influence of 50 % (partial) scale removal on (a) emergence (b) inflorescence height and (c) fresh weight of bulb at harvest of the common hyacinth during the 2006/2007 planting season. Bars represent means ± SE of four replicates.

(a)



(b)



(c)

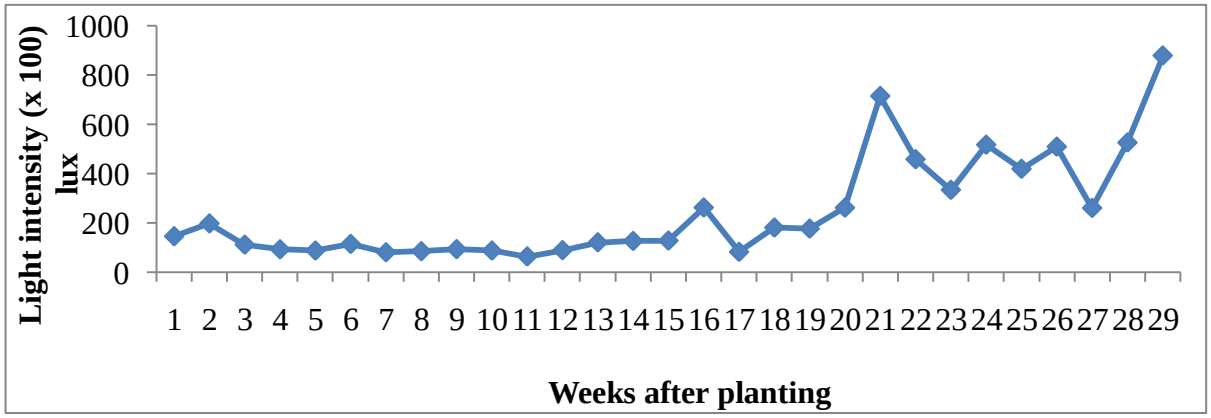
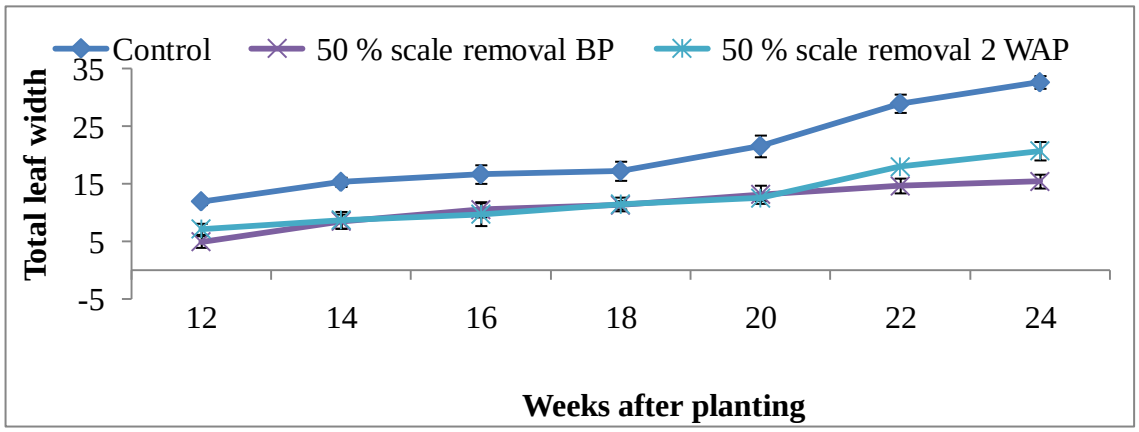


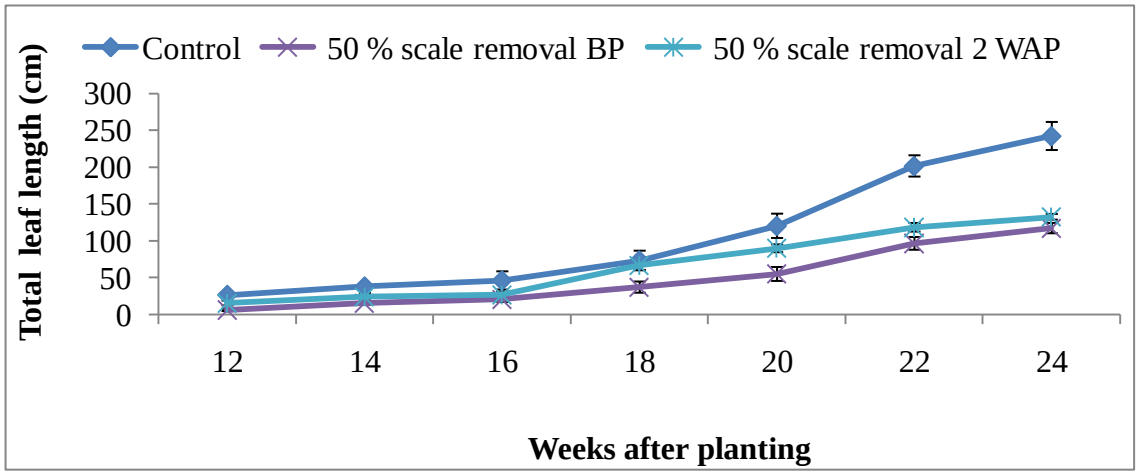
Fig 4.03: Variations in greenhouse (a) temperature (b) relative humidity and (c) light intensity from November 2006 to June 2007. Measurements were made daily in the greenhouse between the hours of 12.00 (mid-day) to 2.00 pm and averages computed for week.

Plants produced from the 100 % peeling treatment before planting recovered in chlorophyll (Fig 4.06b) and their chlorophyll concentration became significantly similar to those produced from bulbs that were 100 % peeled two weeks after planting. Results on the effects of peeling on hyacinth inflorescence development in 2008/2009 were similar to that observed in 2006/2007 because plants from peeled bulbs produced significantly lower inflorescence height than those from the unpeeled control. Additionally, complete (100 %) scale removal treatments reduced inflorescence height more than did the partial (50 %) scale removal treatment (Fig 4.07). Plants produced from the two partial scale removal treatments, which is 50 % peeling prior to planting and at two weeks after planting produced similar inflorescence height. Also, plants produced from bulbs whose scales were completely (100 %) peeled prior to planting did not produce any inflorescence whereas those subjected to the same severity of peeling but at two weeks after planting produced some inflorescence. Inflorescence length, inflorescence stalk diameter, and number of florets (Fig 4.08) all followed similar trends as inflorescence height. Also, as in 2006/2007 season, the fresh weights of bulb at harvest in 2008/2009 from the various peeling regimes followed a similar trend as leaf growth (Fig 4.09).

(a)



(b)



(c)

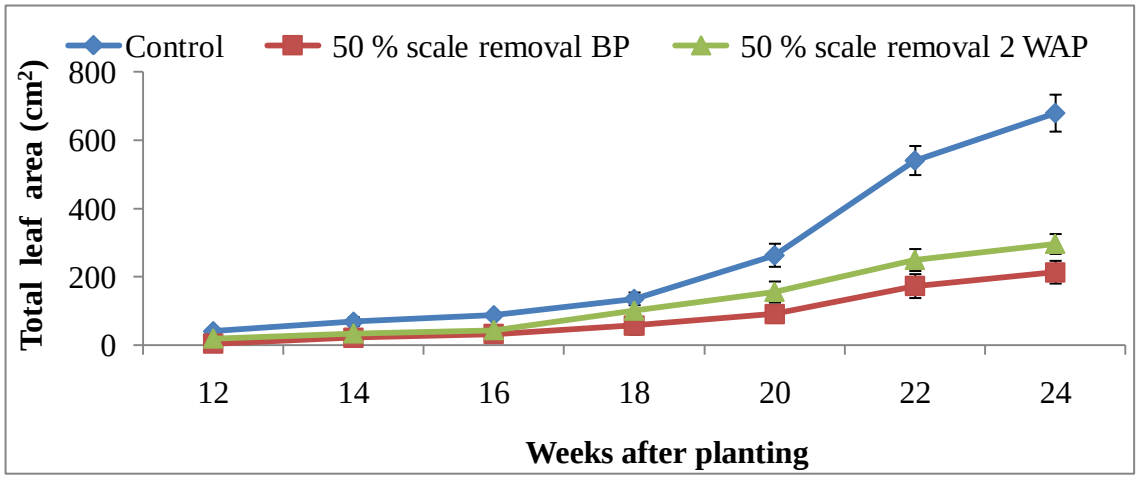
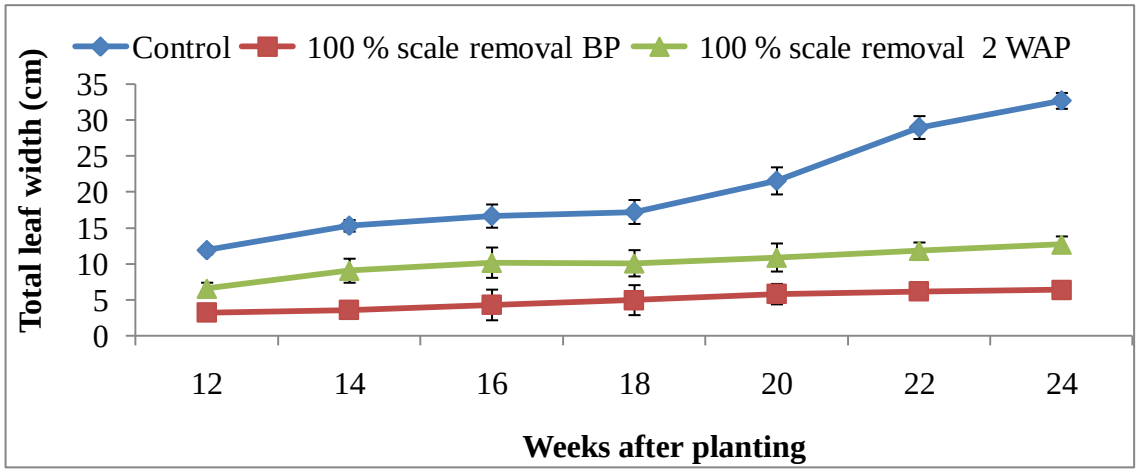
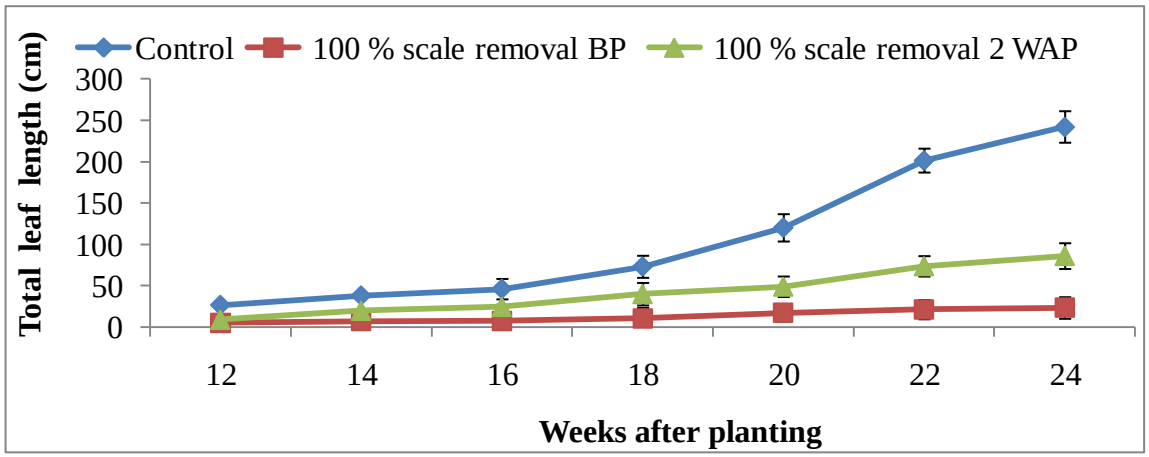


Fig 4.04: Effects of partial (50%) scales removal on leaf growth: (a) leaf width, (b) leaf length and (c) leaf area of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates.

(a)



(b)



(c)

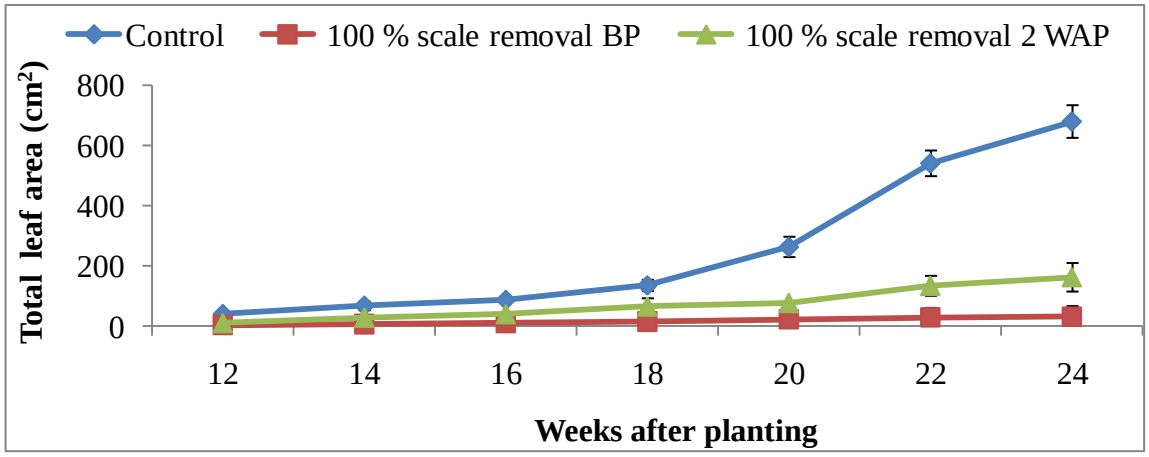
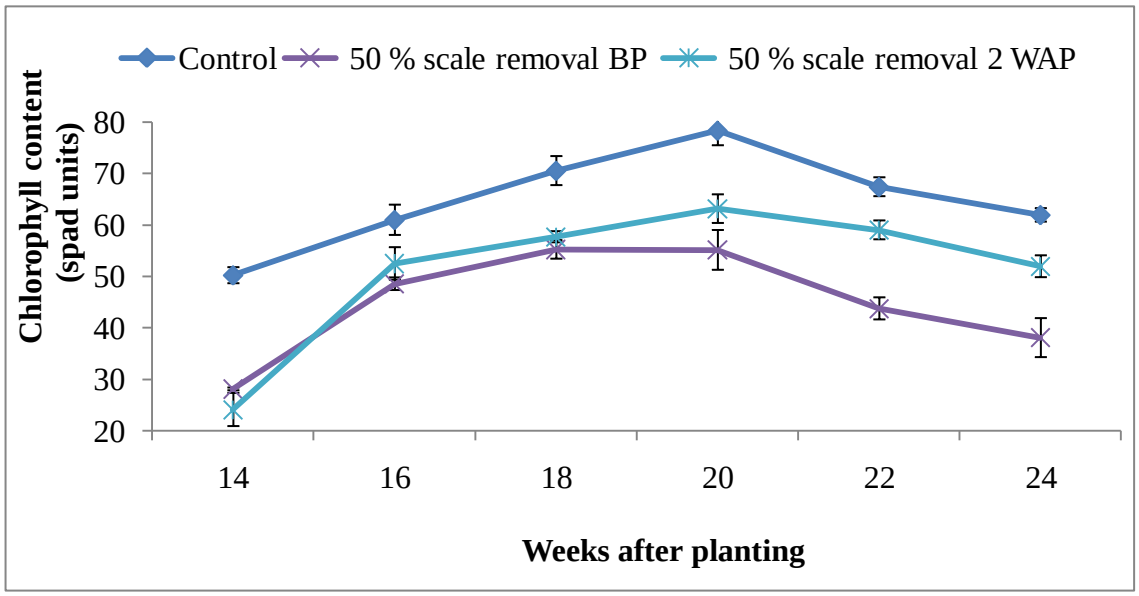


Fig 4.05: Influence of complete (100%) scales removal on leaf growth: (a) leaf width, (b) leaf length and (c) leaf area of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates.

(a)



(b)

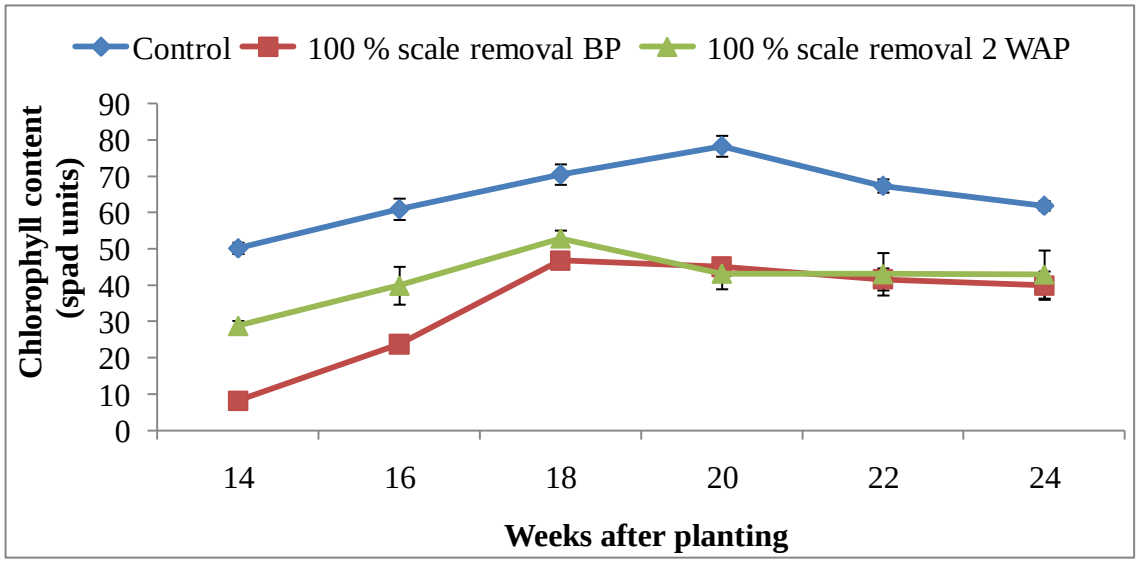
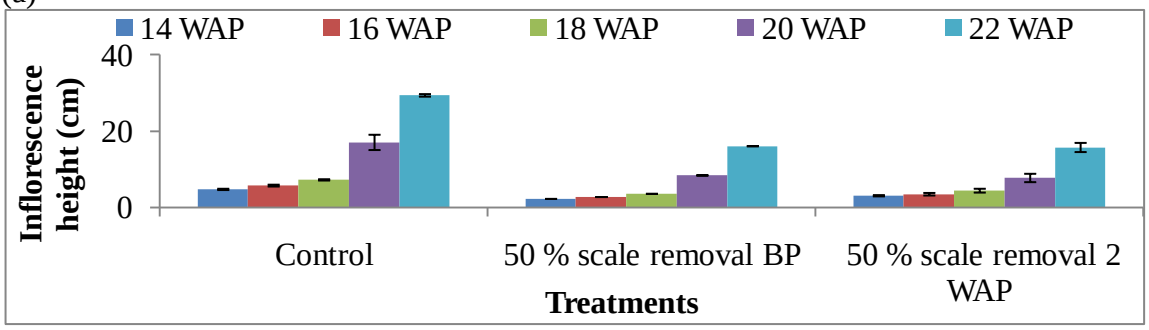
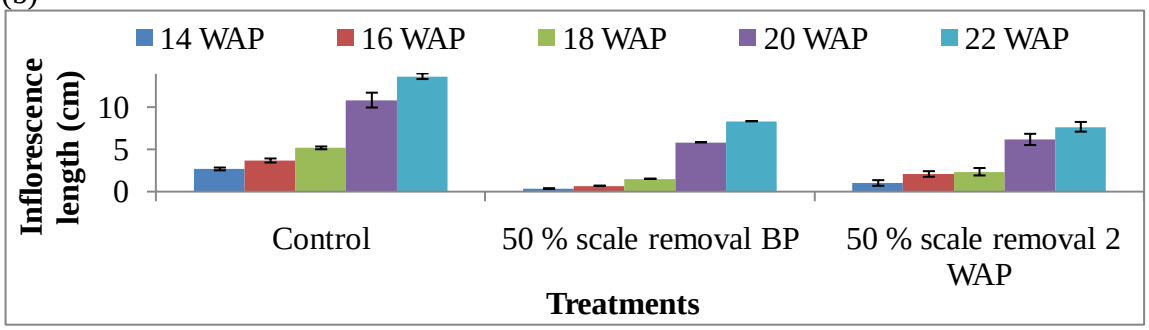


Fig 4.06: Influence of (a) partial (50%) and (b) complete (100%) scales removal on leaf chlorophyll content of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates. During measurements, three points (the lower part, middle section and towards the tip) of each leaf was measured using the chlorophyll meter and averages computed for each plant. Bulbs do not store only reserved food materials or water in their scale leaves, but they also store minerals such as Mg^{2+} ions, which are needed for chlorophyll synthesis in their scales. Thus the removal of the bulb scales as a result of peeling denied the developing plants of these ions that are needed for the synthesis of chlorophyll. This explains why chlorophyll content of plants produced from peeled bulbs was low as compared to plants produced from the unpeeled control.

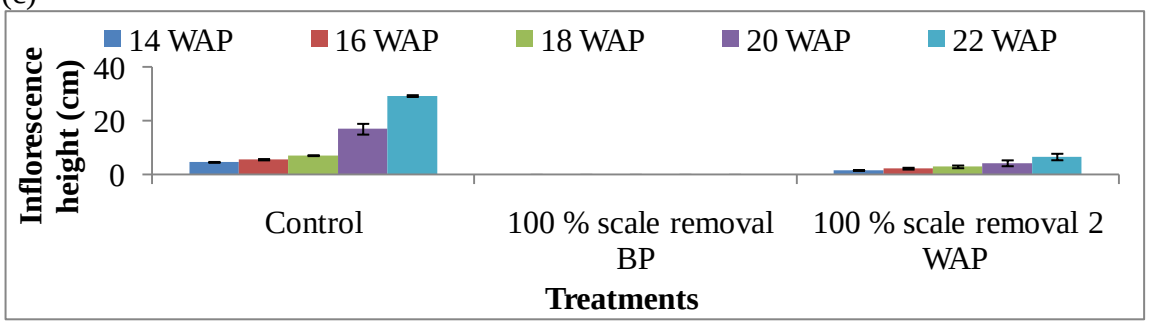
(a)



(b)



(c)



(d)

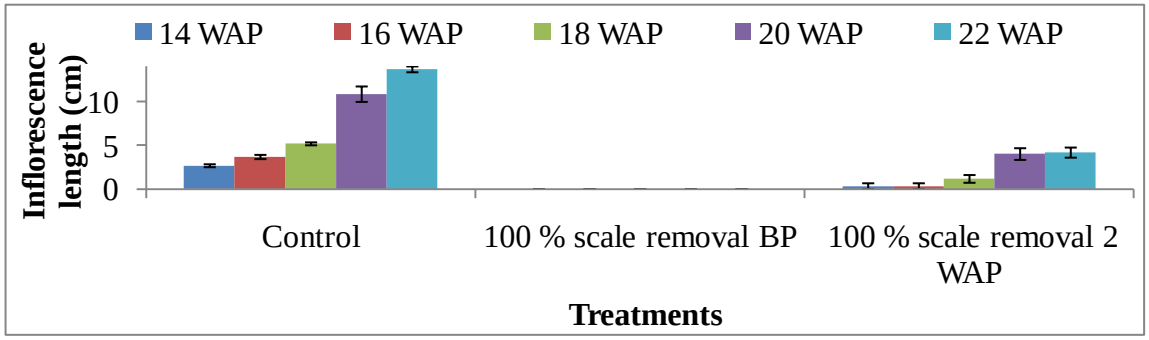
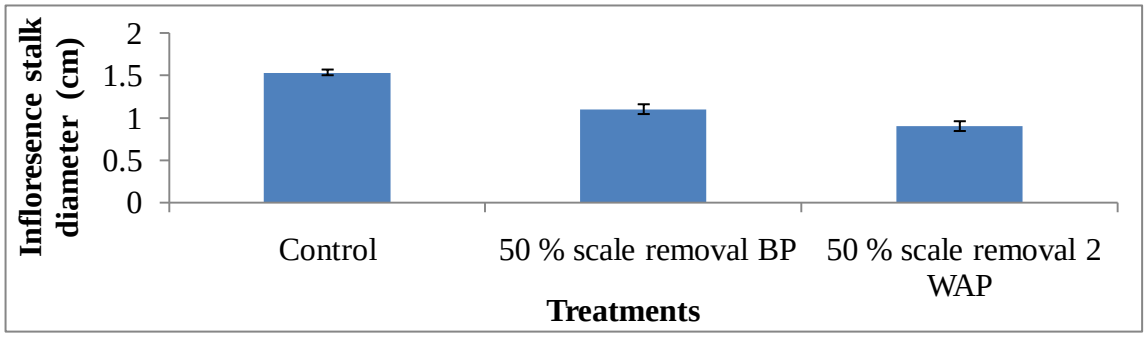
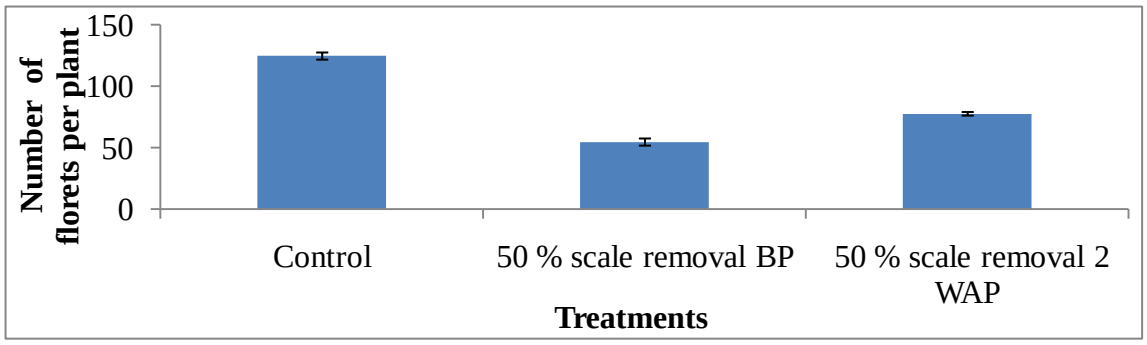


Fig 4.07: Effects of scale removal on hyacinth's inflorescence development: Influence of partial peeling on (a) inflorescence height (b) inflorescence length. Inflorescence growth response to complete scales removal: (c) inflorescence height and (d) inflorescence length of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates. Complete inhibition of inflorescence formation as a result of 100 % scales removal prior to planting of the bulbs suggests that in flower bulbs, reserves are mobilised from the scales for the development of the flower, or elongation of the flower stalk.

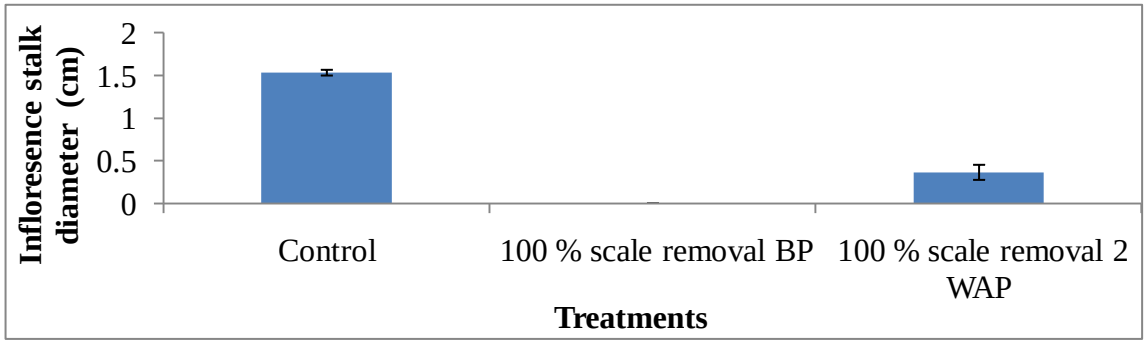
(a)



(b)



(c)



(d)

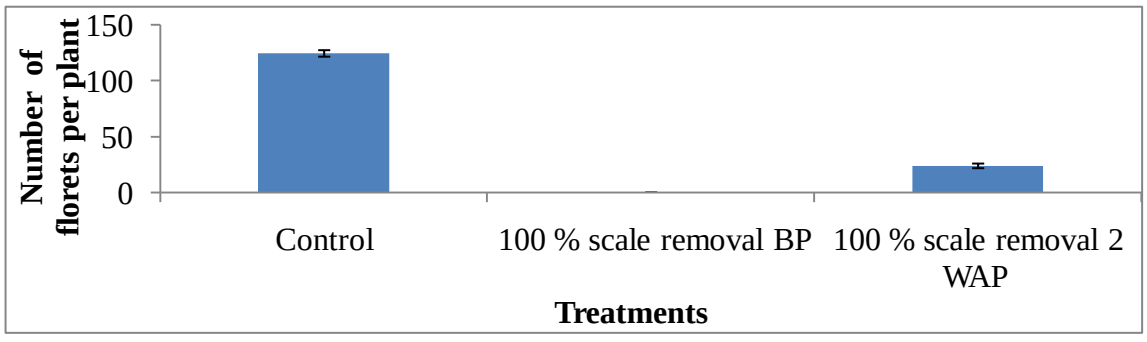
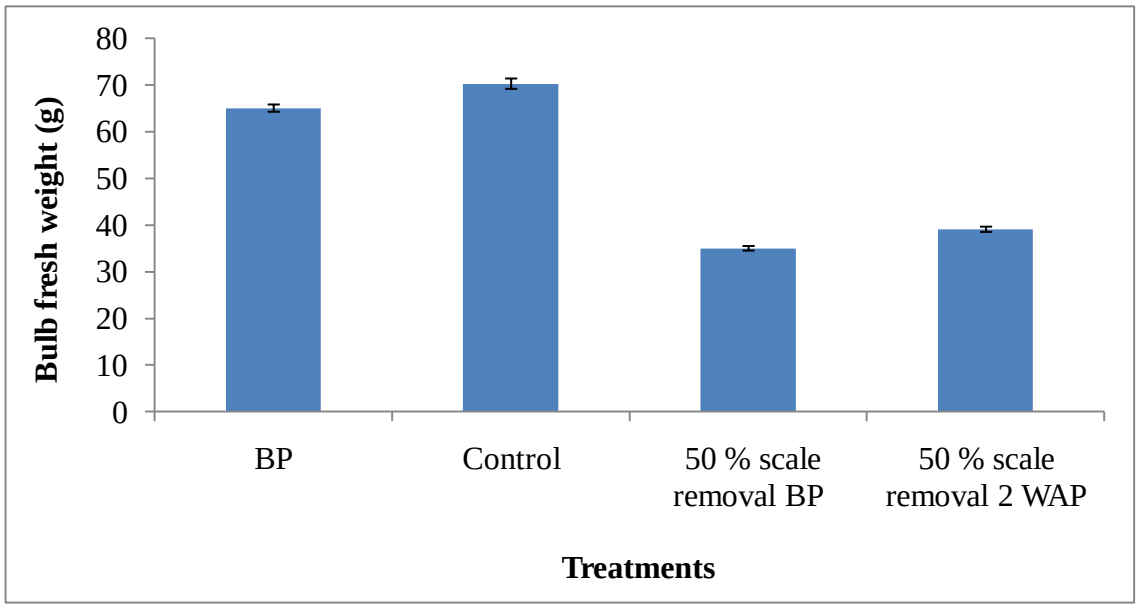


Fig 4.08: Fig 4.07: Effects of scale removal on hyacinth's inflorescence stalk and number of floret, (a) Inflorescence stalk diameter, (b) number of florets and the effects of complete scales removal on (c) Inflorescence stalk diameter and (d) number of florets of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates.

(a)



(b)

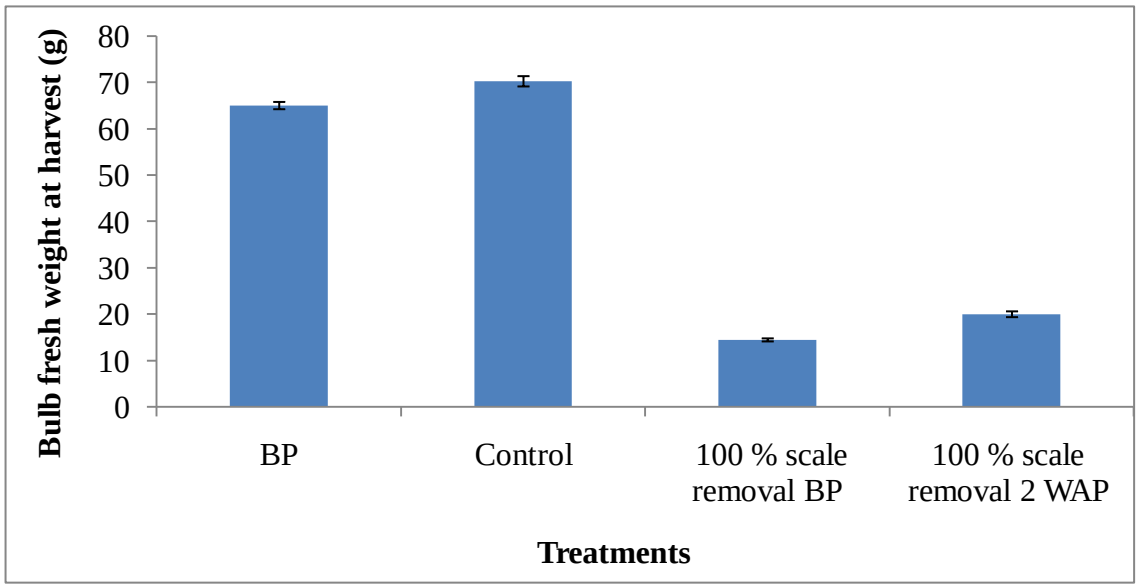
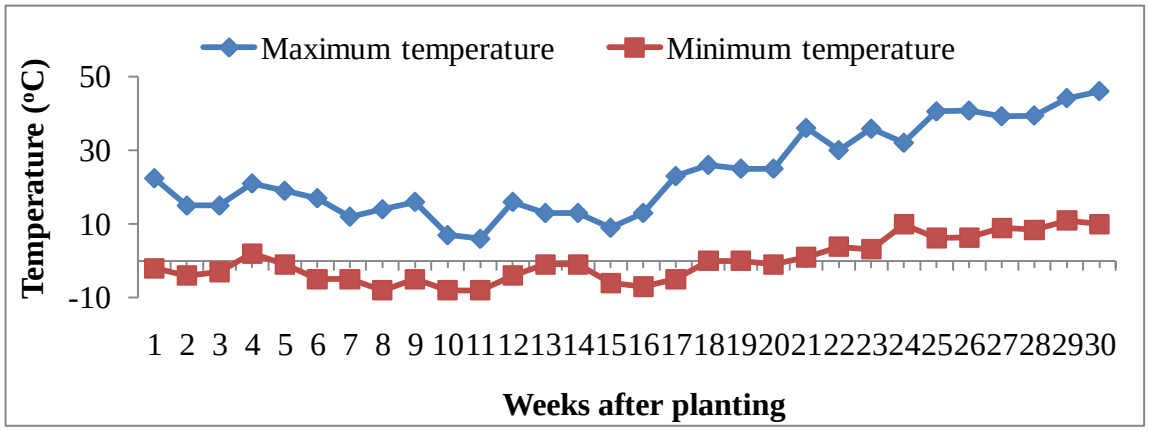
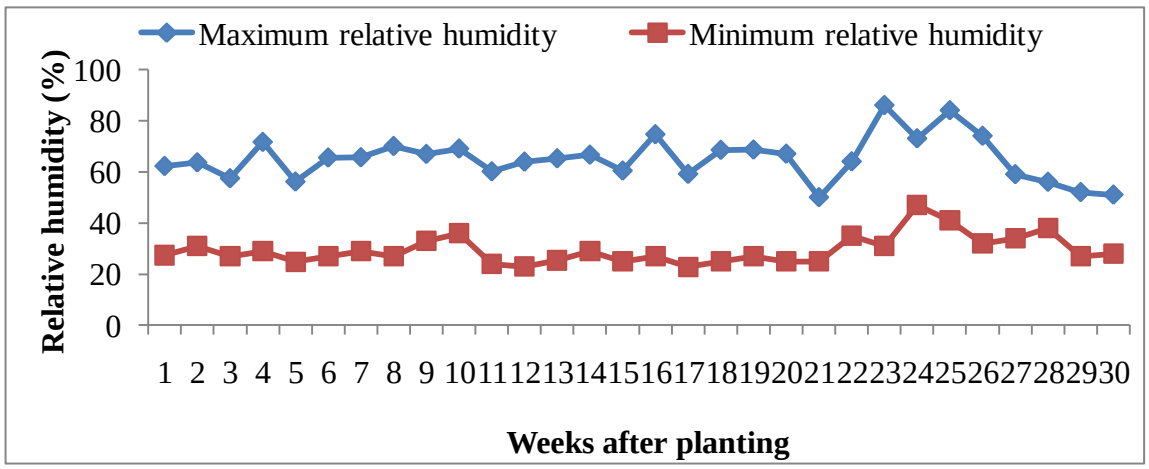


Fig 4.09: Influence of scales removal on fresh weight of bulbs at harvest; (a) effects of partial bulb scale removal and (b) complete bulb scale removal on the bulb fresh weight at harvest of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates.

(a)



(b)



(c)

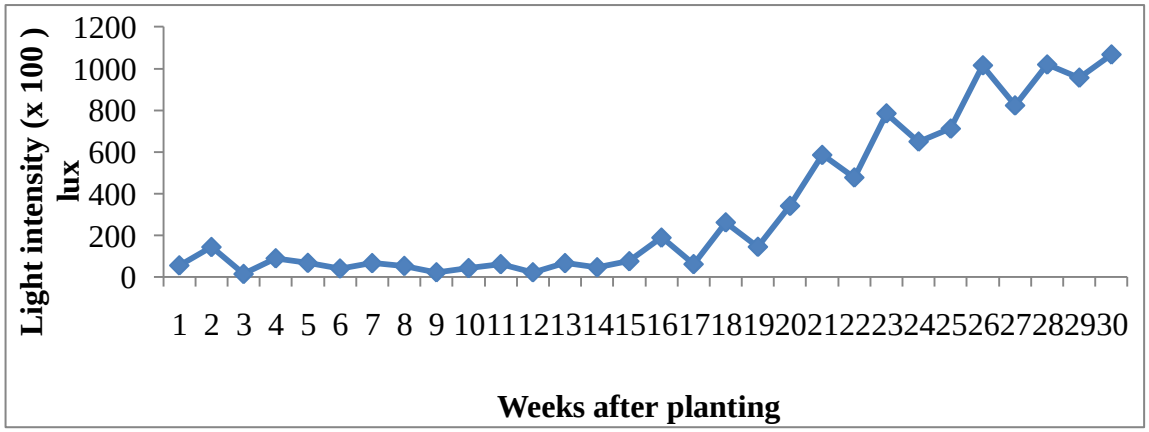
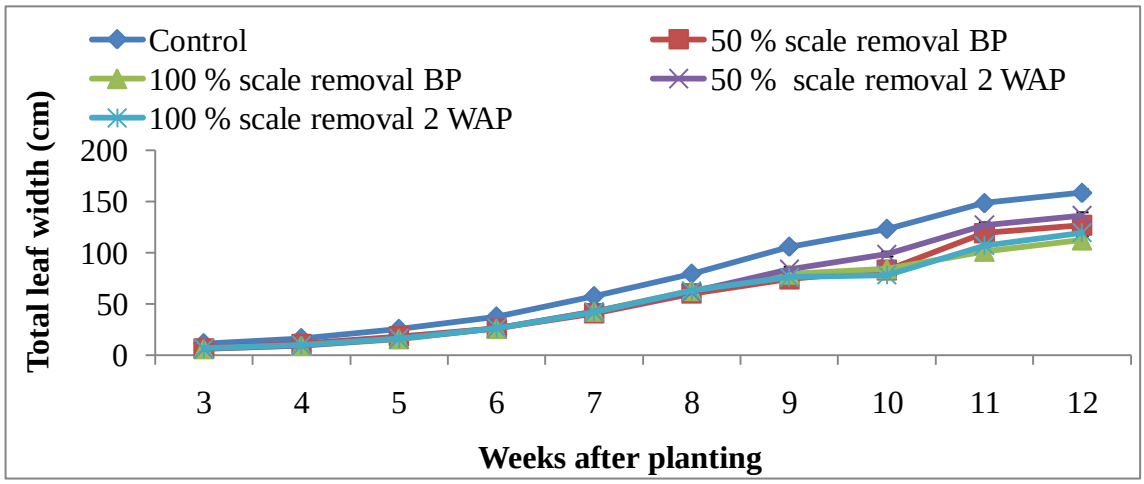


Fig 4.10: Changes in the greenhouse weather parameters during the experimental period from October 2008 to May 2009. Each value represents a mean of seven days measurement in the week, and measurements made each day between the hours of 12.00 and 14.00 GMT.

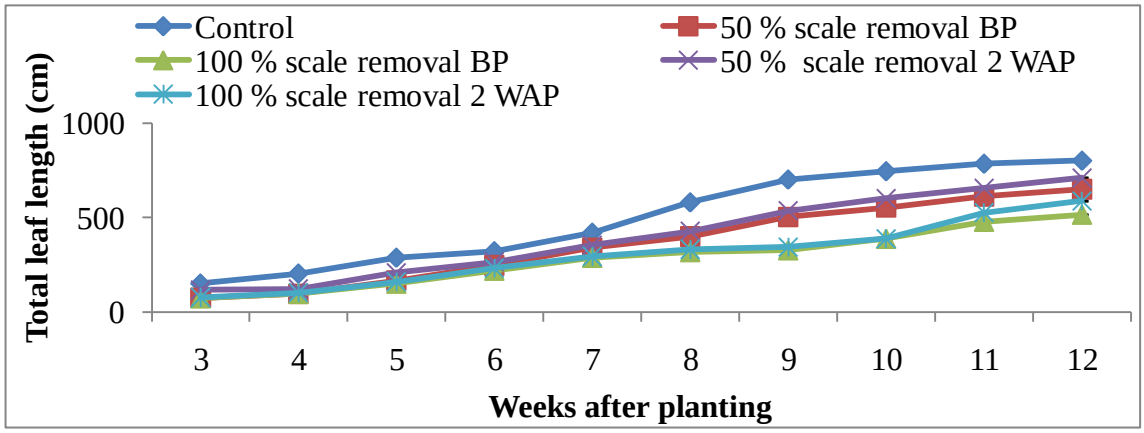
4.2.1.2 The lily

During the 2007/2008 summer periods, Lily bulbs were also subjected to both complete (100 %) scale removal and partial (50 %) scale removal treatments, just like the hyacinth experiment that was conducted during the spring season of the same year. The performances of plants produced from peeled bulbs were compared to their unpeeled control counterparts. Results from the lily experiment were not too different from those of the hyacinth experiment. As usual, parameters measured from plants obtained from all the peeling regimes i.e. leaf growth, plant height, and inflorescence height, chlorophyll content, photosynthetic rate and stomatal conductance decreased relative to those of the control (Fig 4.11 - 4.13). Also, these parameters generally decreased with increasing intensity of the scale removal treatment. That is, plants subjected to complete scale removal had lower values of the measured parameters as compared to those whose bulb scales were partially removed. As it was observed in the case of hyacinth, 100 % peeling of the lily bulbs before planting (BP) completely inhibited inflorescence formation. At harvest, bulbs produced from the 100 % scale removal prior to planting regime had the highest gain in bulb fresh weight (59 % increase in fresh weight gain relative to those of the control) whilst plants from partial bulb scale removal before planting had the least (Fig 4.12a).

(a)



(b)



(c)

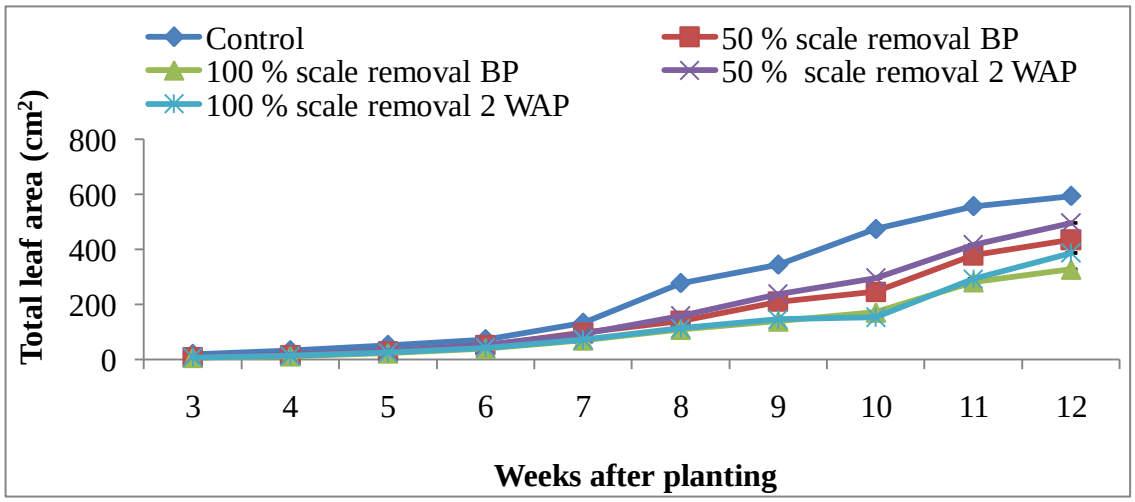
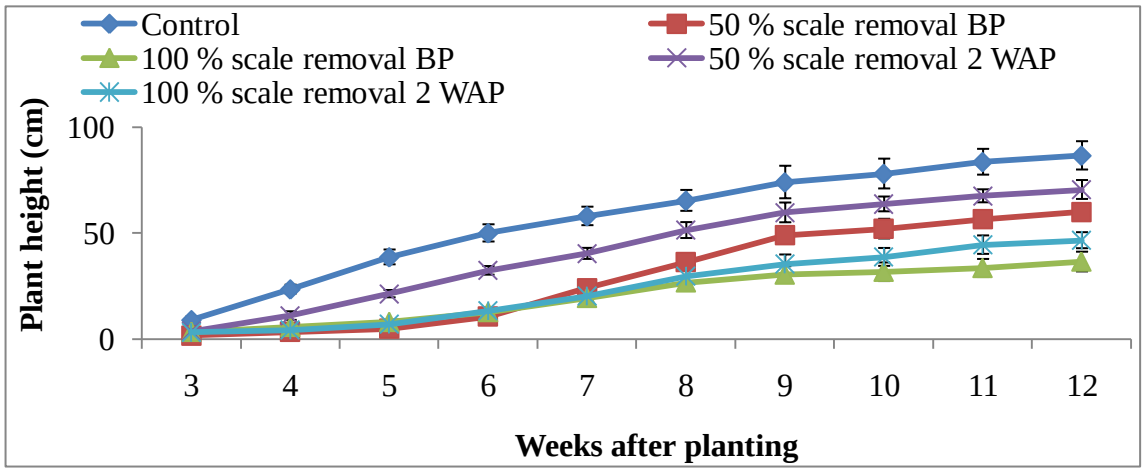
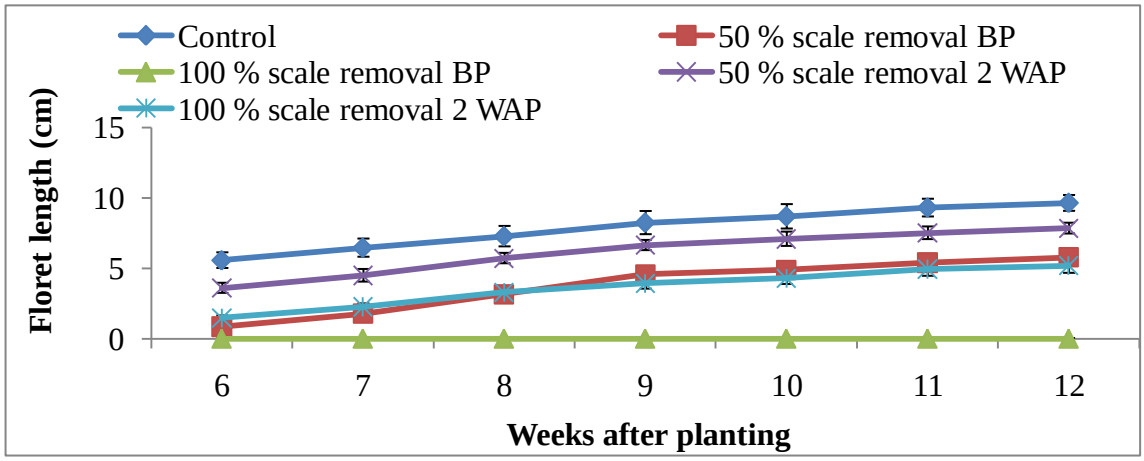


Fig 4.11: Influence of partial or complete scale removal on leaf growth of the lily bulb: (a) leaf width, (b) leaf length and (c) leaf area of the lily. Error bars represent the mean ± SE of four replicates.

(a)



(b)



(c)

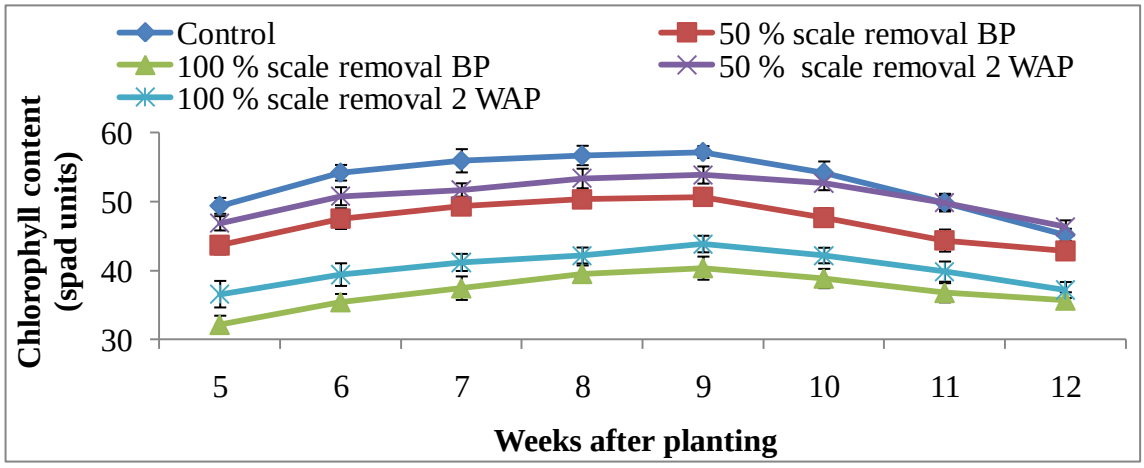
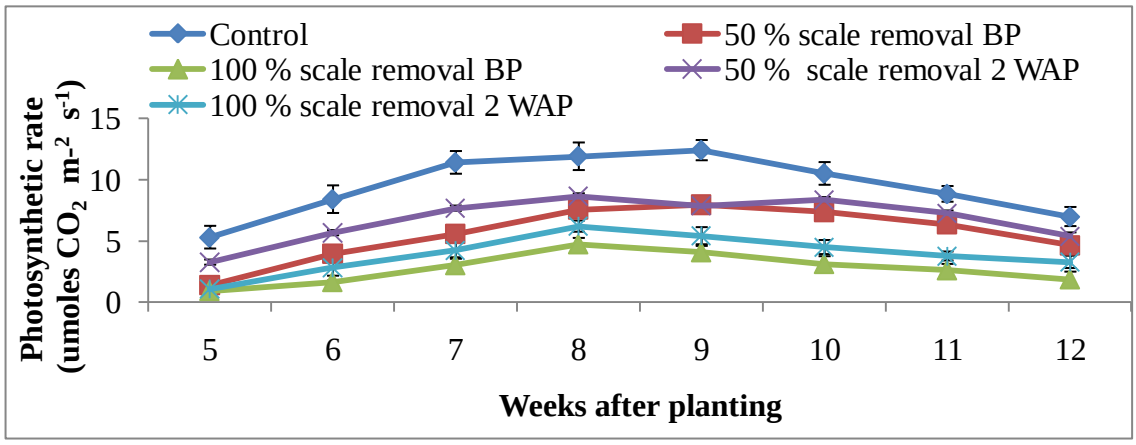
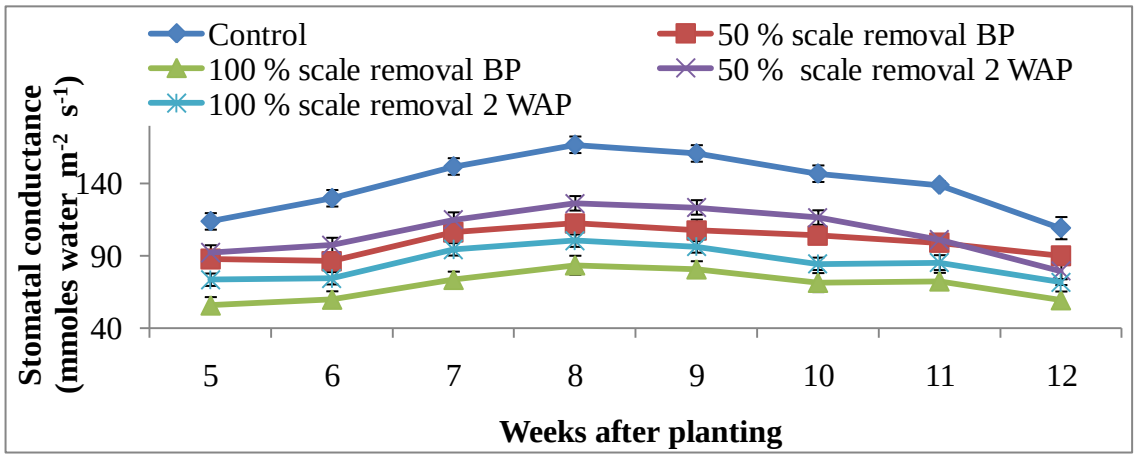


Fig 4.12: Effects of partial or complete bulb scale removal on (a) plant height (b) floret length and (c) chlorophyll content of the lily. Error bars represent the mean ± SE of four replicates.

(a)



(b)



(c)

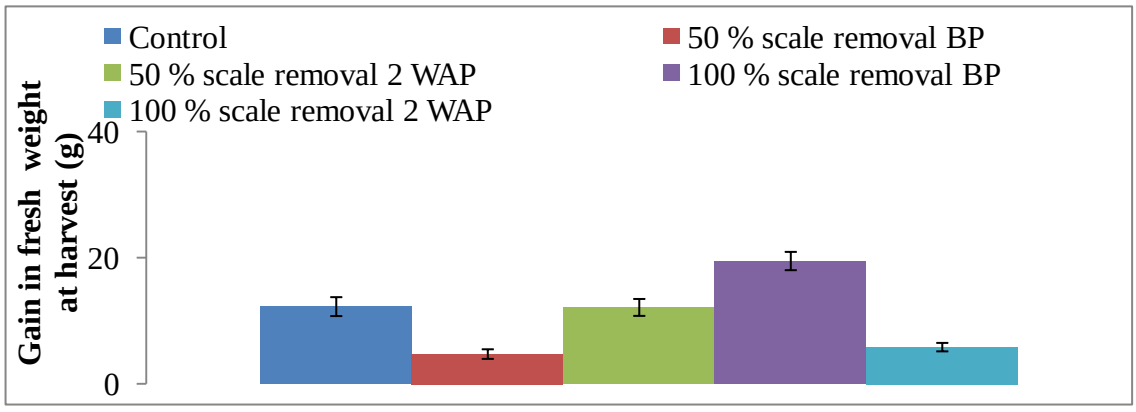


Fig 4.13: Partial or complete bulb scale removal effects on lily: (a) photosynthetic rate (b) stomatal conductance and (c) Weight gain at harvest. The absence of flowers/florets (major sink), as a result of complete (100 %) scale removal before planting of the lily bulb, suggests that assimilates were channelled for bulb development instead of flower formation, hence the highest gain in bulb fresh weight at harvest.

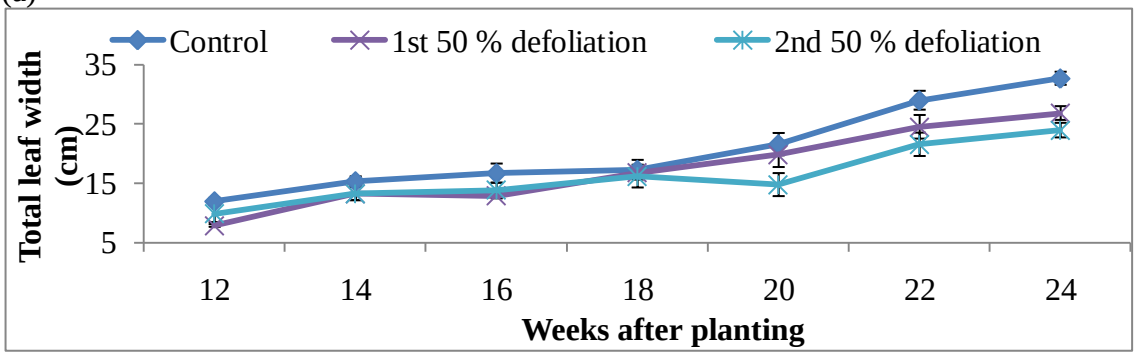
4.2.2 Response of the bulbs to above-ground herbivory

4.2.2.1 Hyacinth

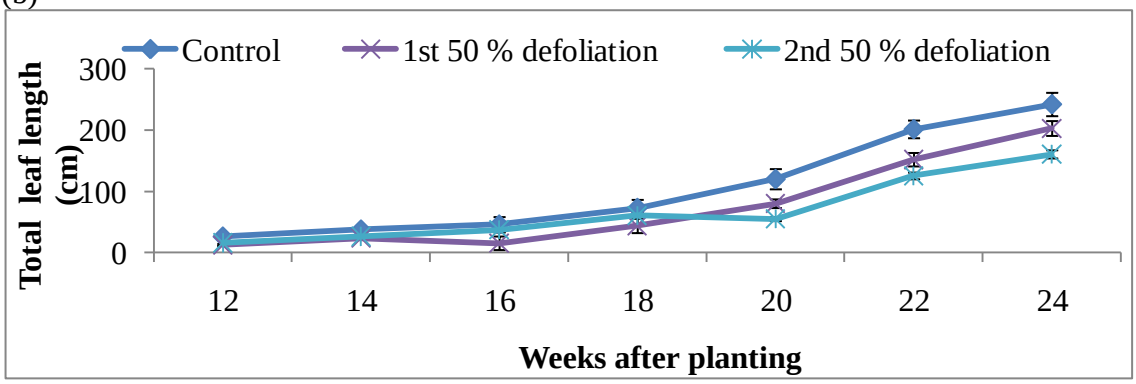
During the planting season of October 2008 to May 2009, another experiment, response of hyacinth to simulated defoliation was also set up. In this experiment, either all leaves (1^{st} 100 % defoliation) or half of each leaf on a plant (1^{st} 50 % defoliation) were manually removed at 14 weeks after planting. Similar leaf removal activities were carried out at 18 weeks after planting (2^{nd} 100 and 50 % defoliations). The performances of plants from these defoliation regimes were compared with those from the undefoliated control and Figure 4.10 shows the influence of greenhouse weather on hyacinth's response to these defoliation activities. Results indicated that both the 50 and 100 % defoliation treatments were detrimental to plant growth as both treatments reduced leaf growth. That is, plants whose leaves were removed as a result of defoliation had lower leaf area values as compared to those from the control (Fig 4.14 and 4.15). Thus, at 24 weeks after planting, total leaf area values of plants belonging to the control, 1^{st} 50 % defoliation and 2^{nd} 50 % defoliation were 679.02 cm^2, 527.23 cm^2 and 377.41 cm^2, respectively, whilst values for the 1^{st} and 2^{nd} 100 % defoliations were 408.22 cm^2 and 230.50 cm^2, respectively. This implies that leaf regrowth ability decreased with increased severity of defoliation because bulbs subjected to 50 % leaf removal produced plants with generally higher leaf area values than those subjected to the 100 % treatment. Also, leaf removal at 14 weeks after planting (1^{st} defoliations) produced plants with higher recovery in terms of vegetative tissues as compared to the second defoliation which took place at 18 weeks after planting in both complete (100 %) and partial (50 %) defoliations treatments. Plants subjected to either the 50 or 100 % leaf removal also had a reduction in leaf chlorophyll concentration because chlorophyll content of defoliated plants were reduced relative to those from the control (Fig 4.16). The 100 % leaf removal treatment reduced chlorophyll content more than did the 50 % defoliation especially at 2-3 weeks after defoliation, but at 24 weeks after planting; the chlorophyll content of the former exceeded that of the latter. In general, all defoliated plants, later recovered in chlorophyll development at 24 weeks after planting. Defoliation also seemed to have had an effect on inflorescence development. Plants whose leaves were completely removed (100 %) at 14 weeks after planting recorded slightly but significantly higher values of inflorescence height and length than those from the control at 22 weeks after planting (Fig 4.17). However, plants from the 50 %

defoliation treatments had inflorescence height and length values that were not significantly different from the control. Moreover, both the 50 and 100 % defoliation treatments reduced fresh weight of bulbs at harvest (Fig 4.18) but the latter had a more detrimental effect on this parameter than did the former, and again, the distribution exhibited by this parameter followed a similar trend as was the case with leaf area.

(a)



(b)



(c)

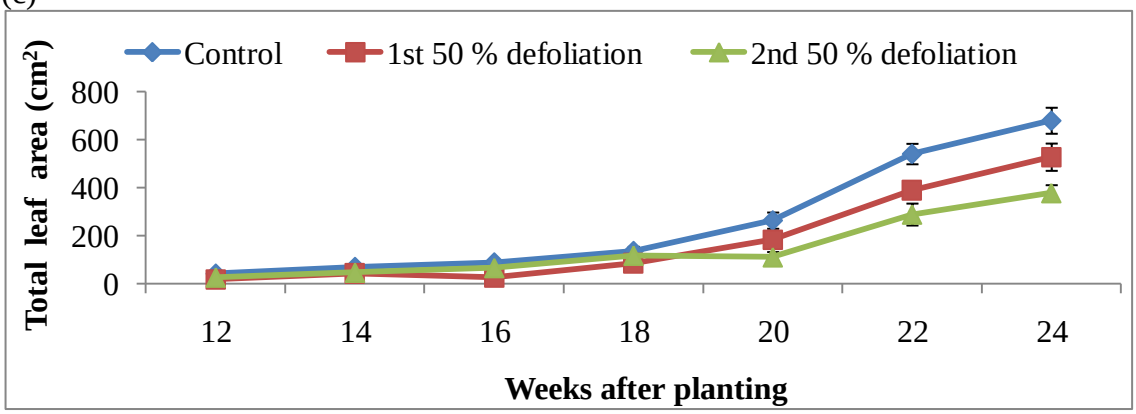
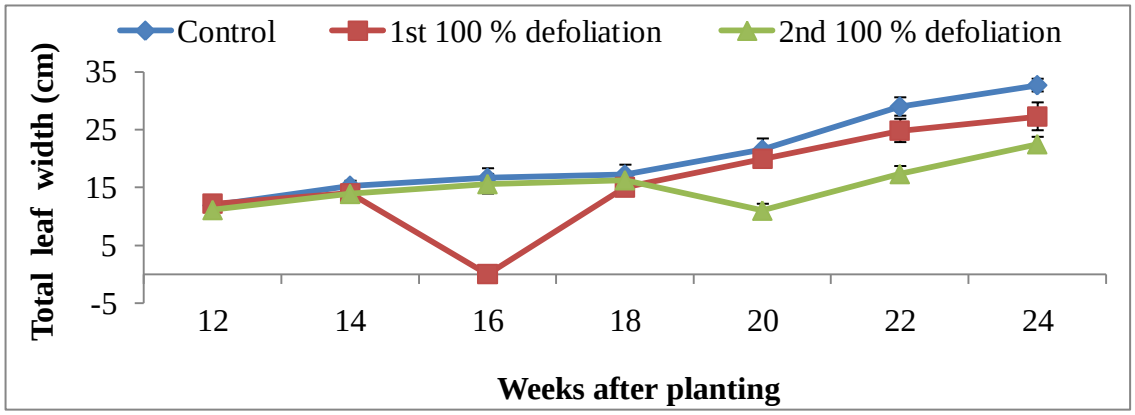


Fig 4.14: Influence of partial (50%) defoliation on leaf growth: (a) leaf width, (b) leaf length and (c) leaf area of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates. Half of all leaves on each plant were manually removed at 14 and 18 WAP, respectively, representing the 1st and 2nd 50 % defoliations.

(a)



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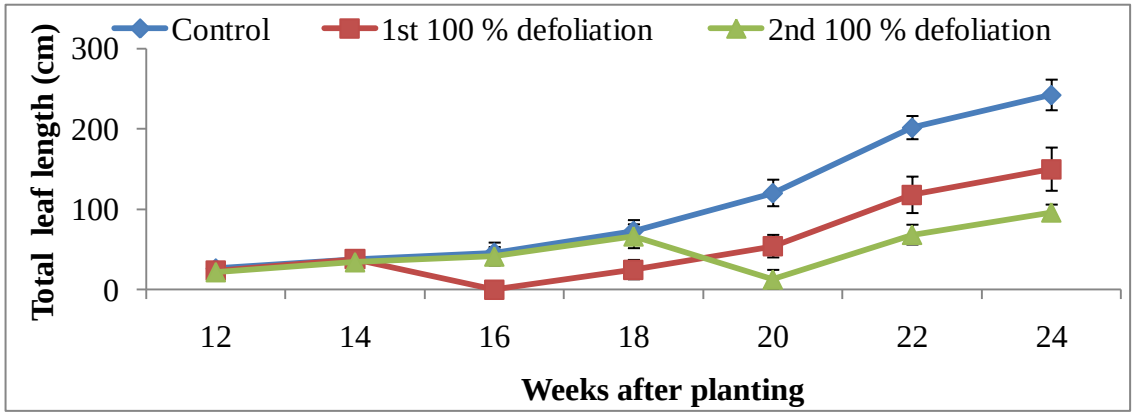

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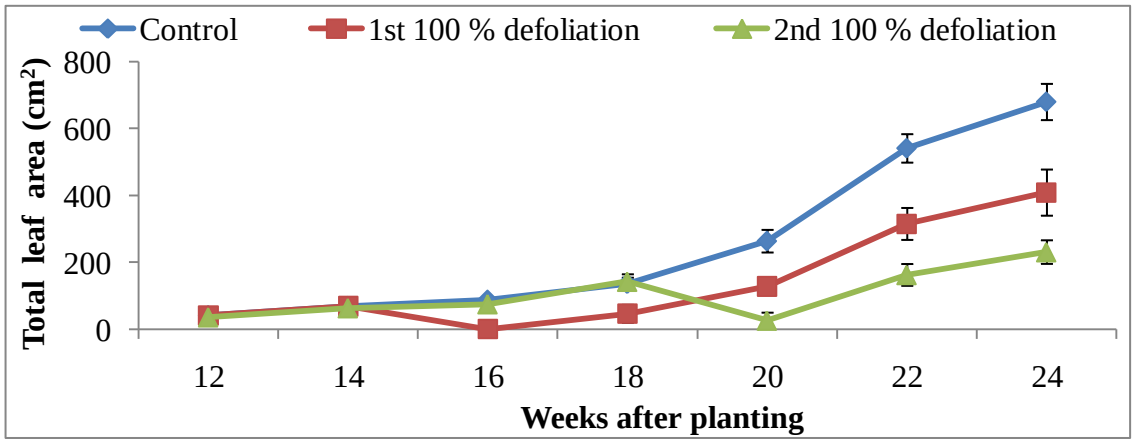
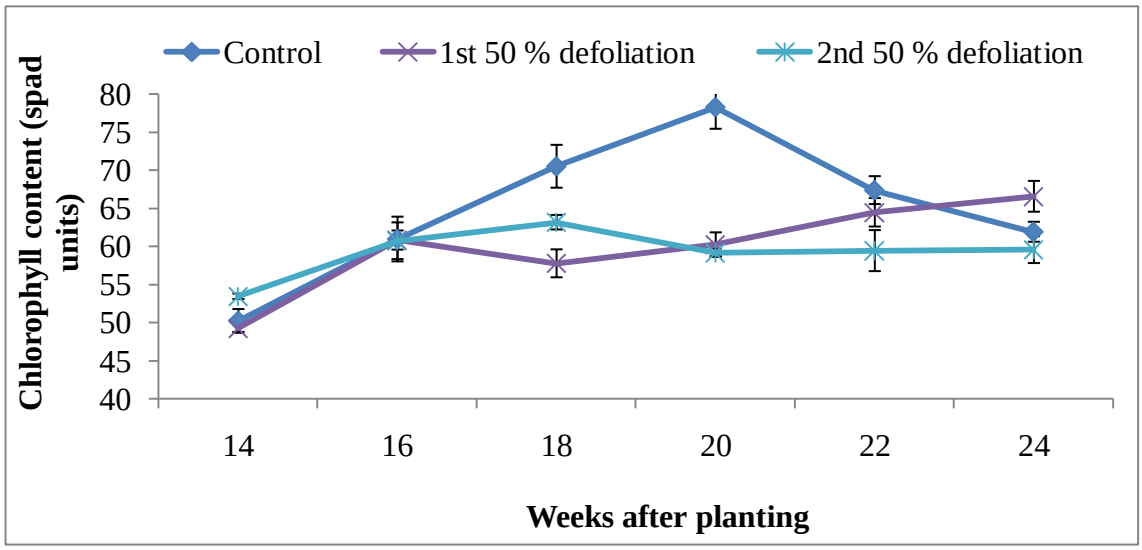


Fig 4.15: Influence of complete (100%) defoliation on leaf growth: (a) leaf width, (b) leaf length and (c) leaf area of the common hyacinth during the 2008/2009 planting season. Leaf removal was conducted at 14 and 18 WAP, respectively, for the 1st and 2nd 100 % defoliation respectively. The error bars represent means ± SE of four replicates.

(a)



(b)

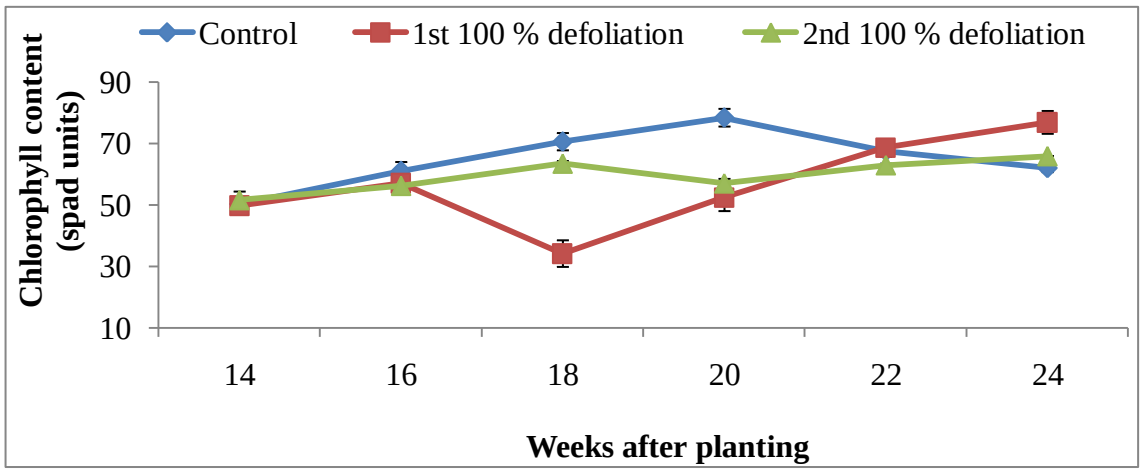
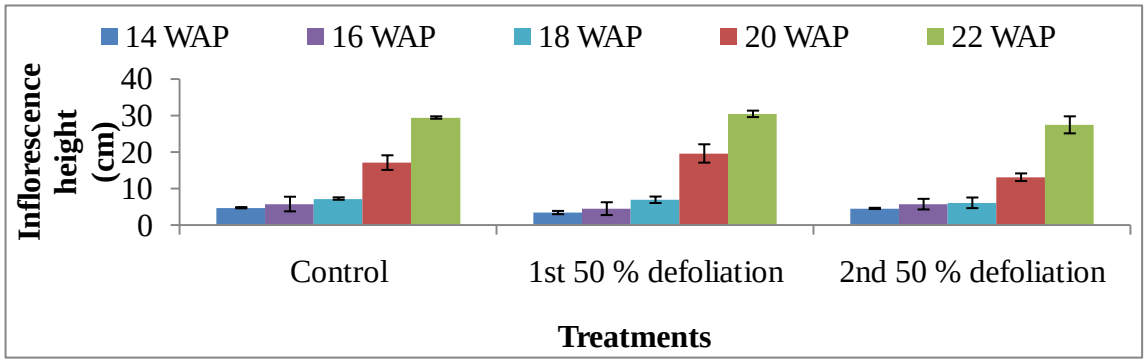
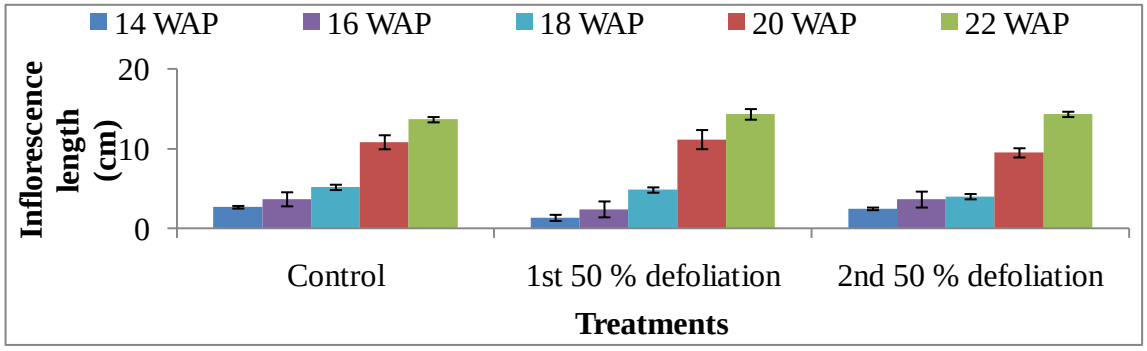


Fig 4.16: Influence of (a) partial (50%), and (b) complete (100%) defoliations on chlorophyll content of the common hyacinth during the 2008/2009 planting season. Three sections (lower portion, middle part and towards the tip) of each leaf in a plant were considered for measurement. Bars represent means ± SE of four replicates.

(a)



(b)



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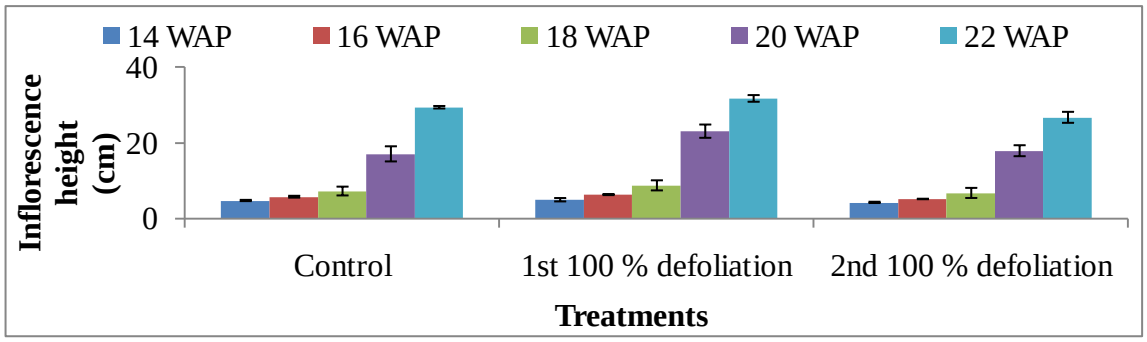


(d)

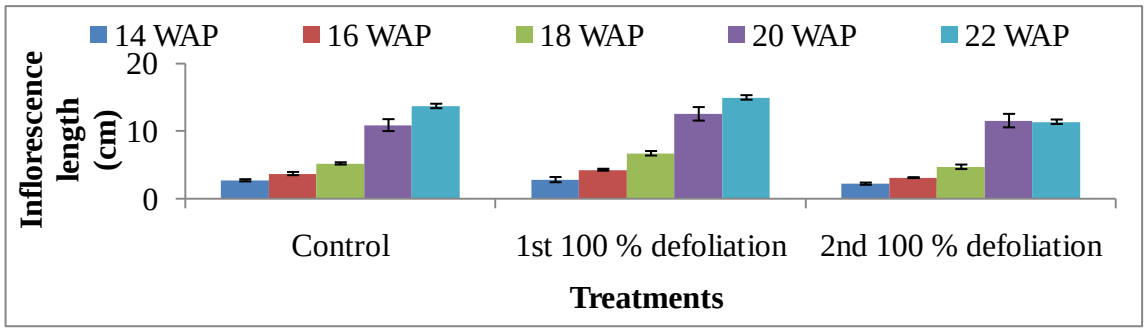
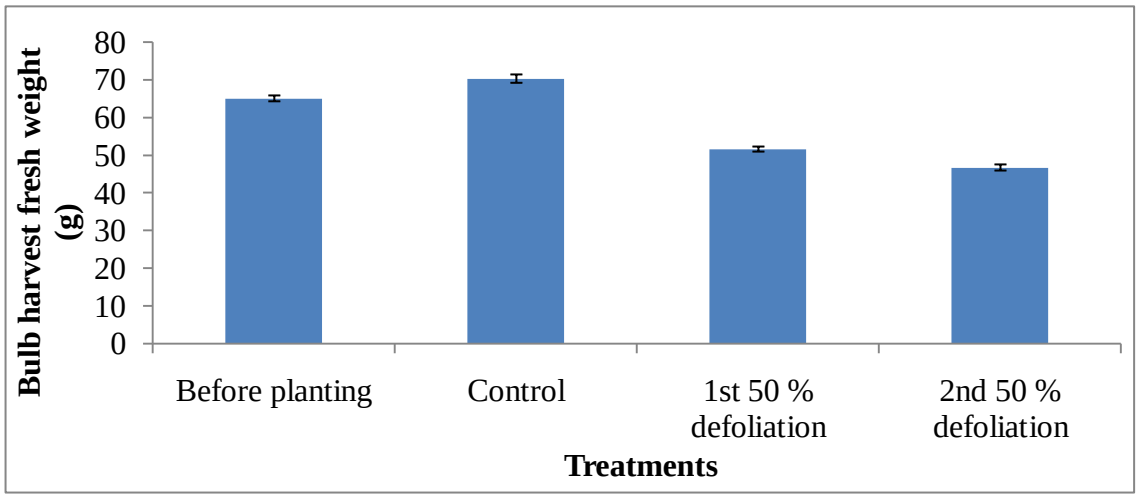


Fig 4.17: Influence of partial defoliations (a) on inflorescence height, and (b) inflorescence length; Influence of 100% defoliation on (c) inflorescence height, (d) inflorescence length, of the common hyacinth during the 2008/2009 planting season. Bars represent means ± SE of four replicates.

(a)



(b)

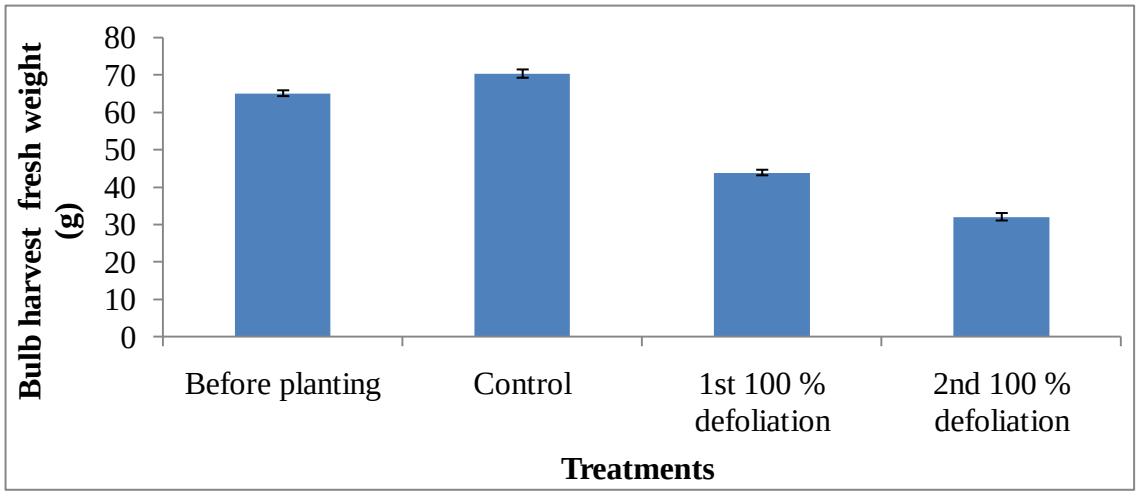
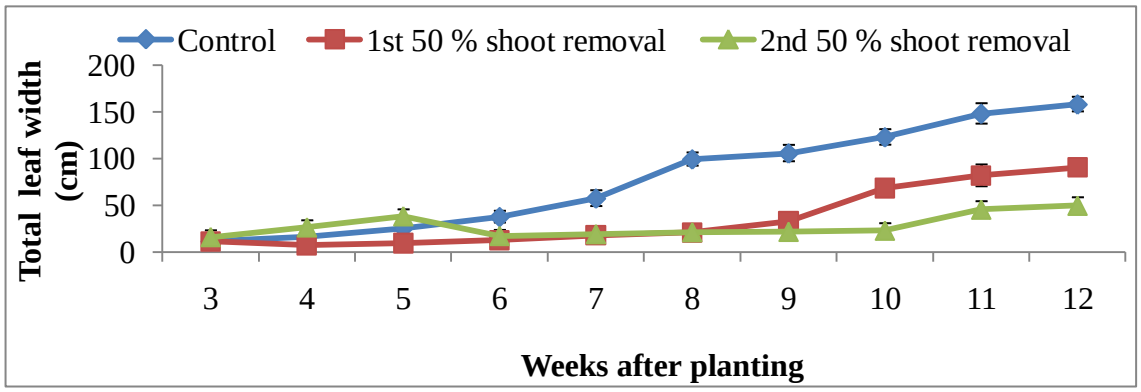


Fig 4.18: Influence of (a) partial defoliation and (b) complete defoliation on fresh weight at harvest of hyacinth bulbs during the 2008/2009 planting season. Bars represent means ± SE of four replicates.

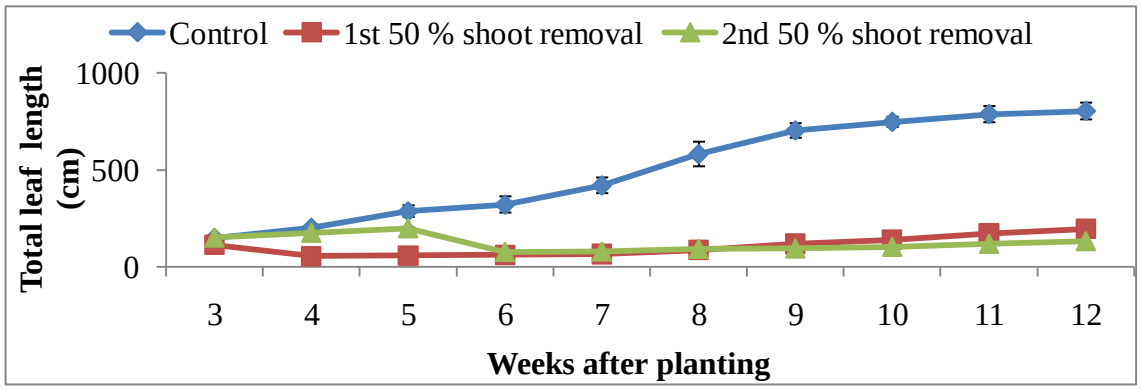
4.2.2.2 The lily

During the summer period of April to August 2008, lily bulbs were also planted and subjected to simulated shoot herbivory. At 3 weeks after planting, either the whole shoot or half the shoot system of each plant were artificially removed (1st 100 and 50 % shoot removal respectively). Similar shoot removal activities were carried out at 5 weeks after planting (2nd 100 and 50 % shoot removal). Changes in temperature, relative humidity and light intensity in the green house during this period are as shown in Figure 4.24. Response of the lily plants to 50 % shoot herbivory in terms of vegetative regrowth (Fig 4.19 – 4.20) was similar to that of 50 % leaf herbivory of hyacinth. Results from the 100 % shoot removal treatment, however, was quite different from those observed in hyacinth because lily plants from the 1st 100 % shoot removal regime produced leaves with high regrowth such that the total leaf length and area of such leaves at 12 weeks after planting were similar to those of the control plants (Fig 4.20a and 4.20b). Similarly, chlorophyll content, photosynthetic rate and stomatal conductance of plants subjected to the 1st 100 % shoot removal treatment were all significantly higher than those of the control (Fig 4.21- 4.22). These parameters, when measured from plants belonging to the 2nd 100 % shoot removal regime, were lower in magnitude relative to those of the control. Gain in bulb fresh weight (Fig 4.23) also followed a similar trend as those of leaf herbivory of hyacinth.

(a)



(b)



(c)

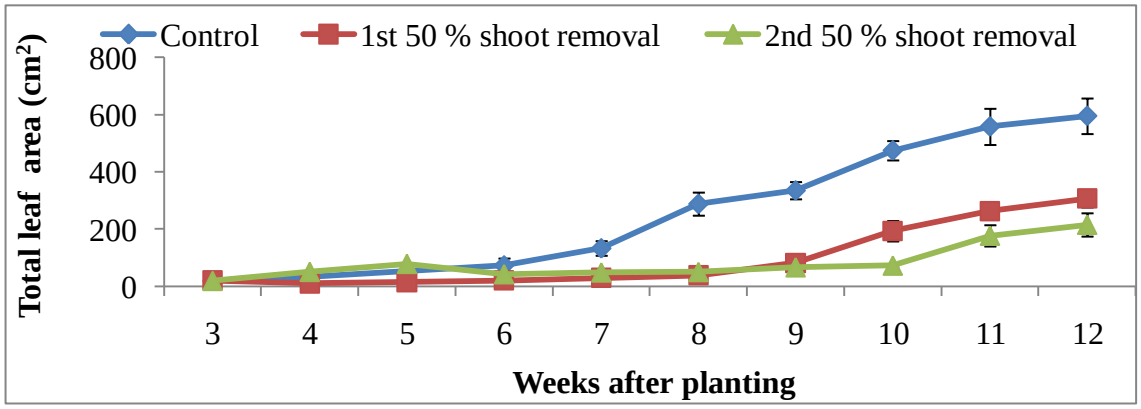
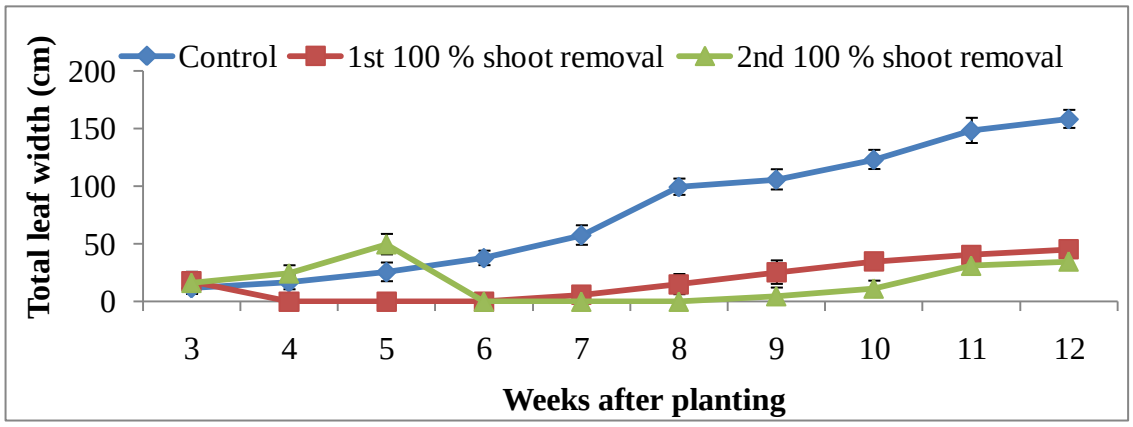
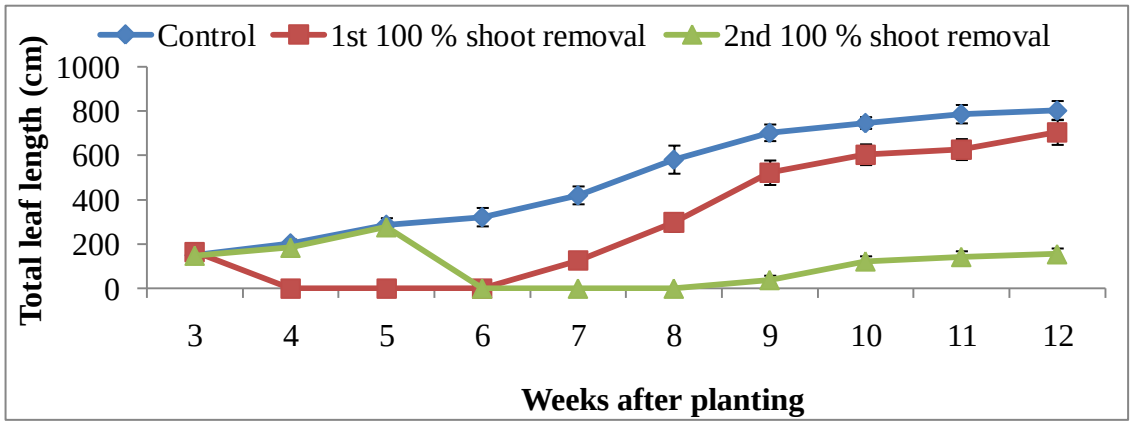


Fig: 4.19: Influence of partial (50%) shoot removal on leaf growth: (a) leaf width, (b) leaf length and (c) leaf area of the lily during the summer season of year 2008. At 3 and 5 weeks after planting (WAP), half the shoots system of each plant was manually removed representing 1st and 2nd shoot removal respectively. Error bars represent means ± SE of four plants.

(a)



(b)



(c)

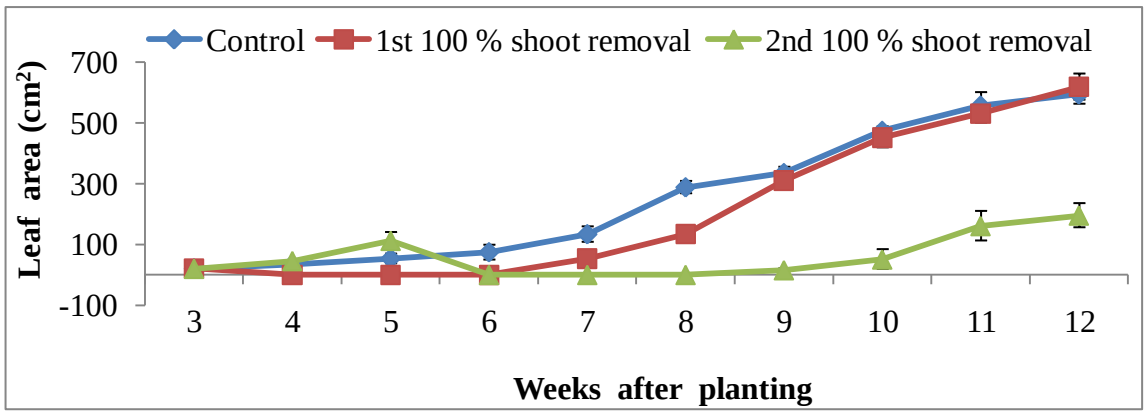


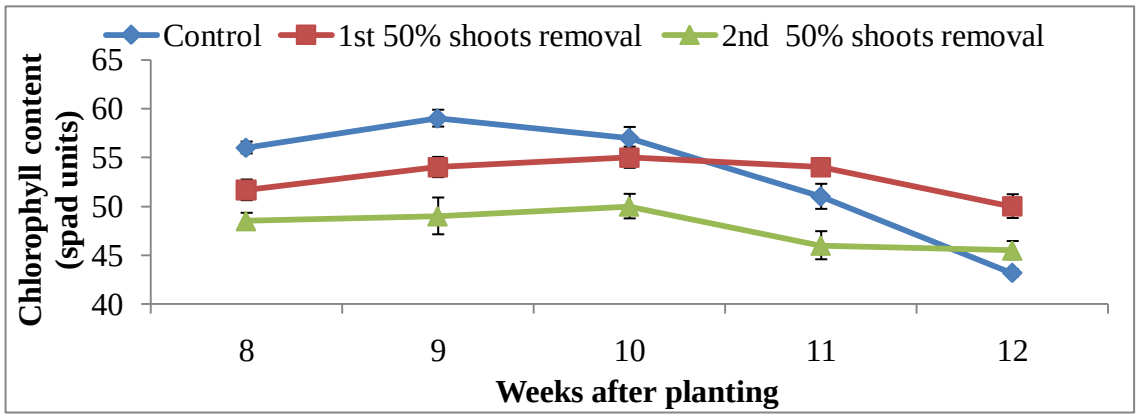
Fig: 4.20a: Influence of complete (100 %) shoot removal on leaf growth: (a) leaf width, (b) leaf length and (c) leaf area of the lily during the summer season of year 2008. At 3 and 5 WAP, the whole shoots system of each plant was manually removed representing 1st and 2nd shoot removal respectively. Error bars represent means ± SE of four plants.

(a)

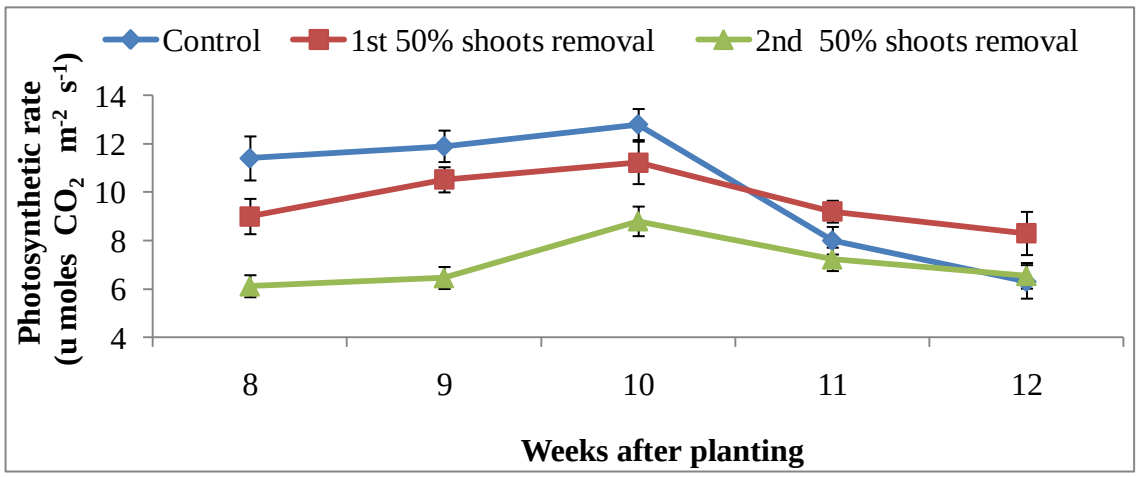
(b)

Fig: 4.20b: Influence of complete (100 %) shoot removal on leaf growth of the lily during the summer season of year 2008: (a) Photograph of plants whose shoots were completely (100 %) removed at 3 WAP (1st 100 % shoot removal), and (b) Plants whose shoots were not removed, i.e. the control. Pictures were taken of these plants at 12 weeks after planting.

(a)



(b)



(c)

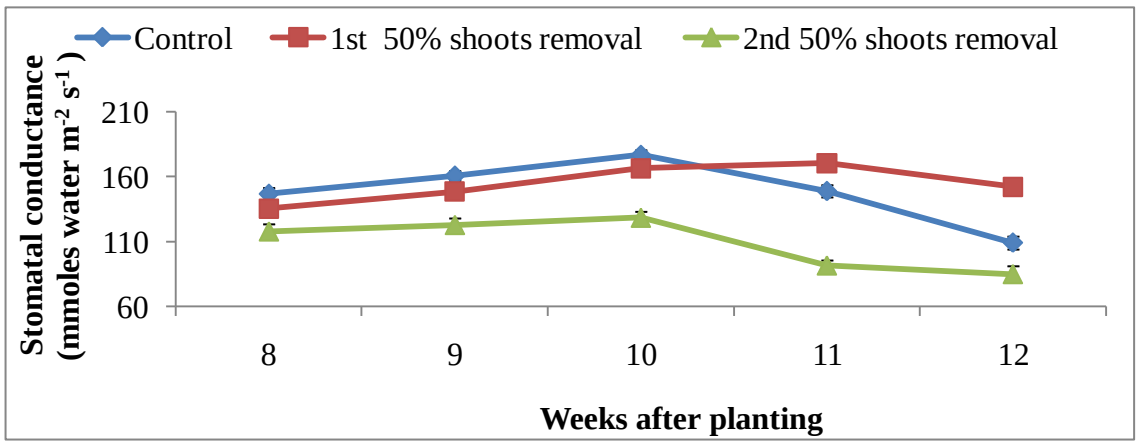
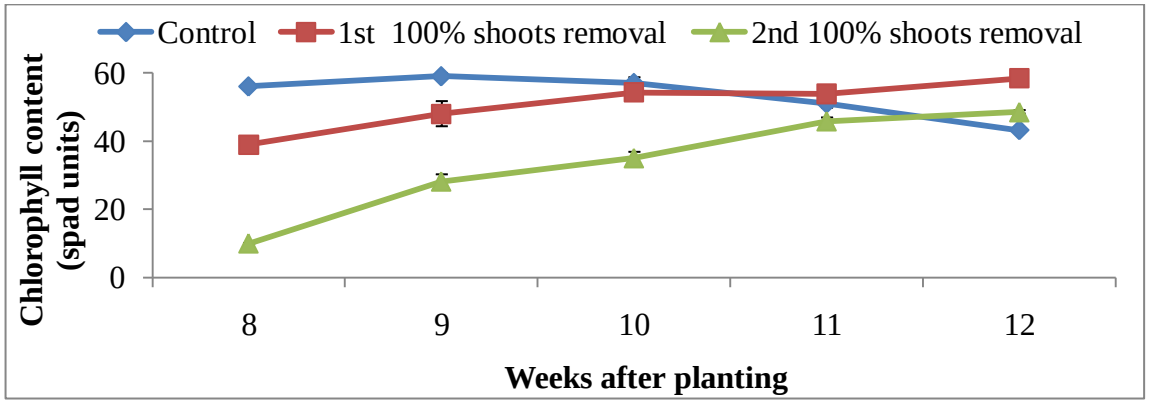
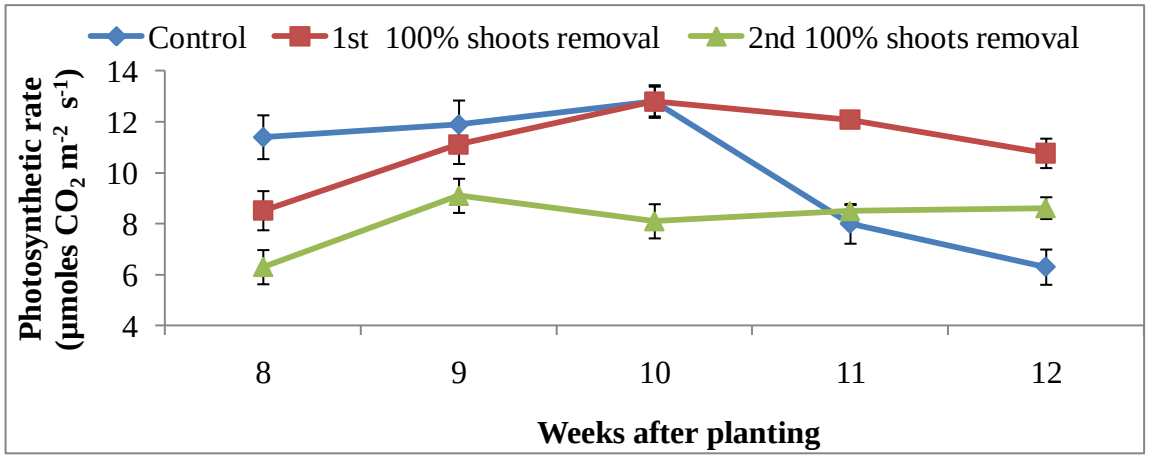


Fig: 4.21: Influence of partial (50%) shoot removal on: (a) chlorophyll content, (b) photosynthetic rate, and (c) stomatal conductance of the lily during the summer period of year 2008. Error bars represent means ± SE of four plants.

(a)



(b)



(c)

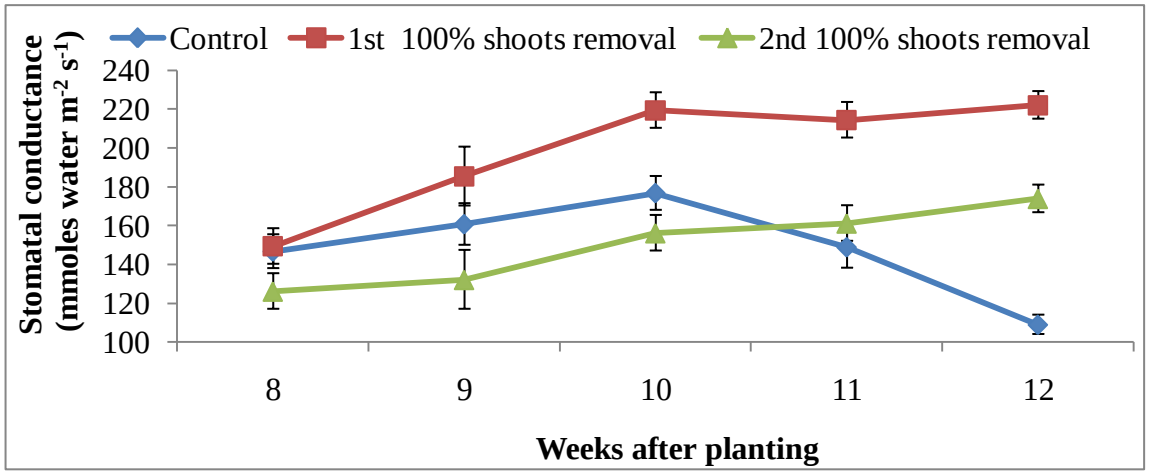
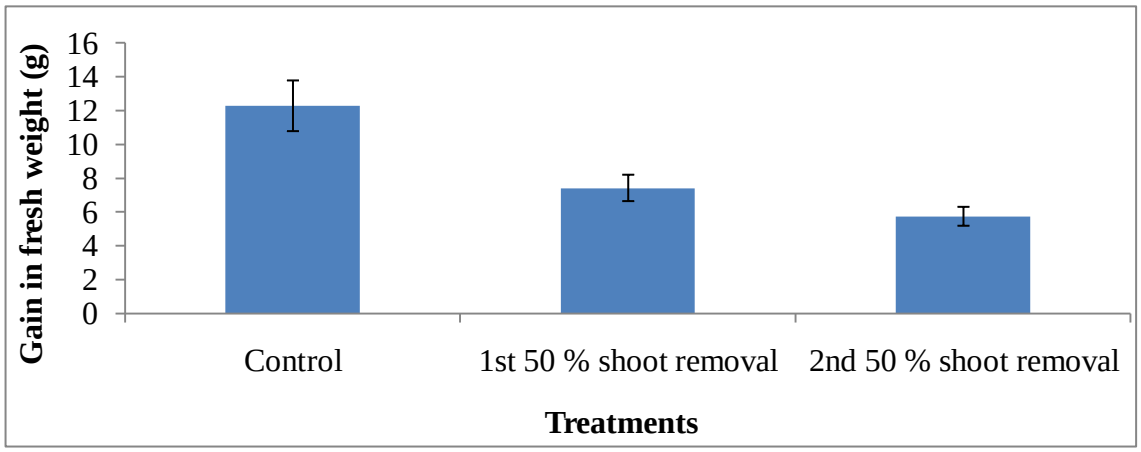


Fig: 4.22: Fig: 4.21: Influence of complete (100%) shoot removal on: (a) leaf chlorophyll content, (b) photosynthetic rate, and (c) stomatal conductance of the lily during the summer period of year 2008. Error bars represent means ± SE of four plants.

(a)



(b)

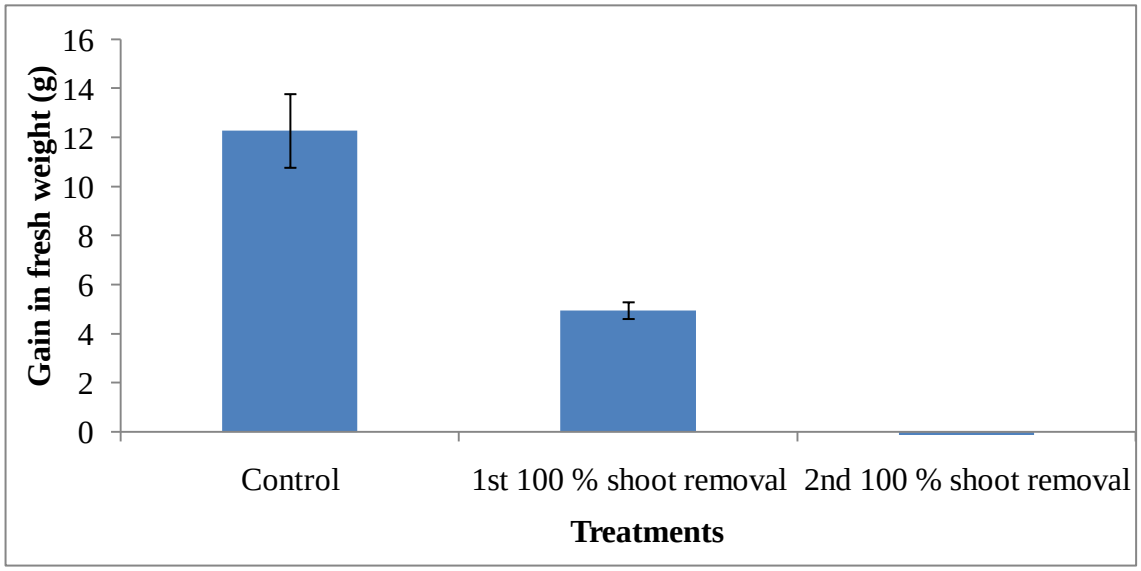
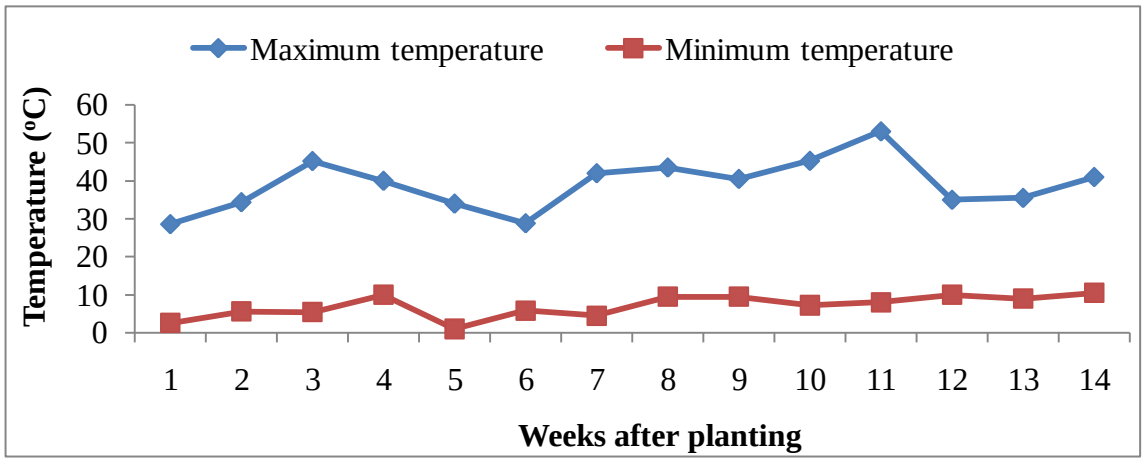
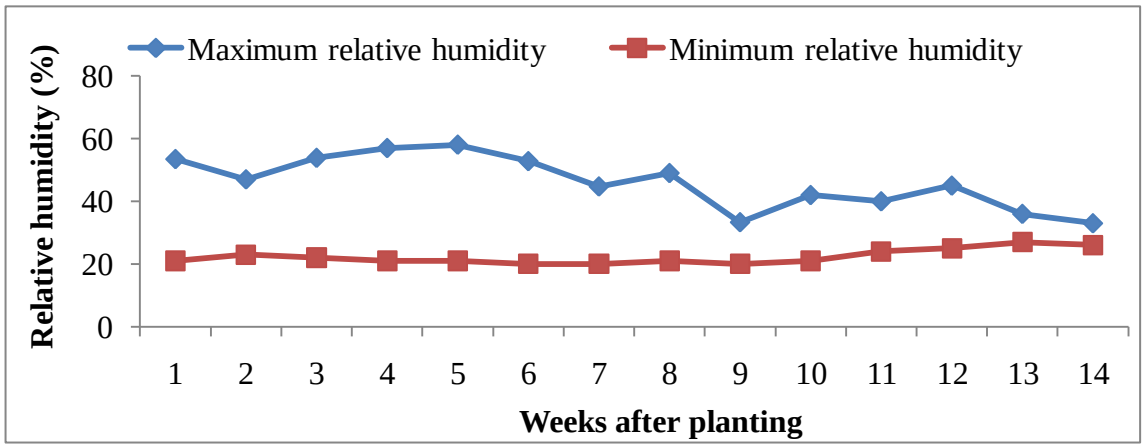


Fig: 4.23: Gain in fresh weight of bulbs at harvest in response to shoot removal of lily during the summer time of year 2008. Weight gain in response to (a) partial shoot removal and (b) complete shoot removal. Error bars represent means ± SE of four plants.

(a)



(b)



(c)

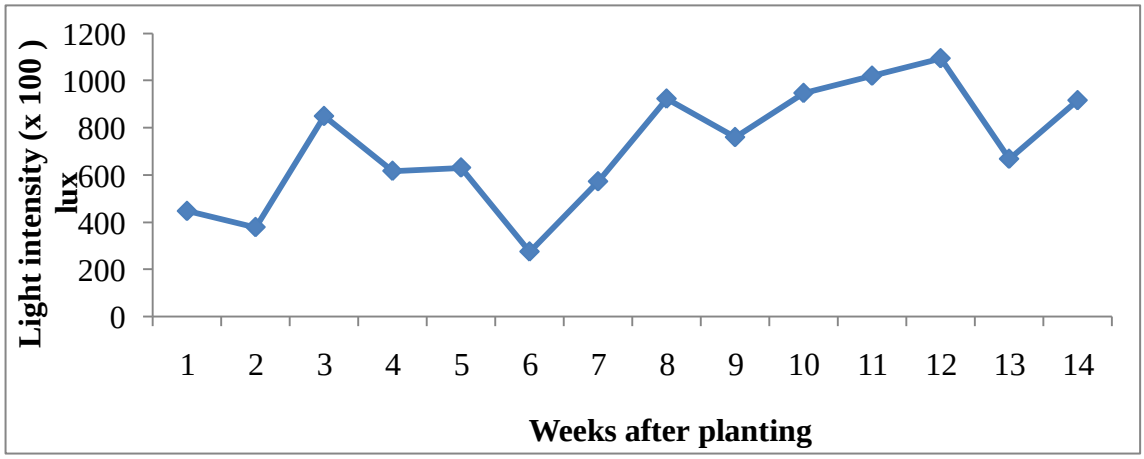


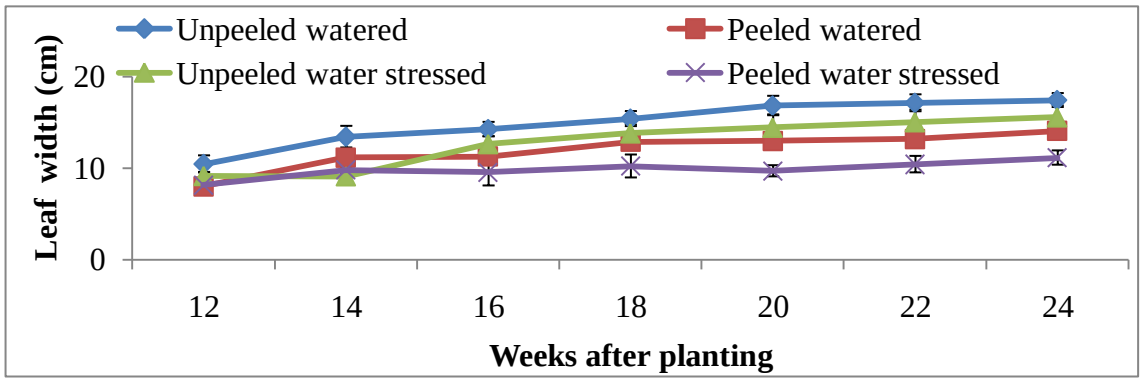
Fig 4.24: Changes in the greenhouse weather parameters during the experimental period from April – August 2009. Each value represents a mean of one week of seven days measurement, and measurements were made each day between the hours of 12.00 and 14.00 GMT.

4.2.3 Effects of drought stress and scale removal on growth and development

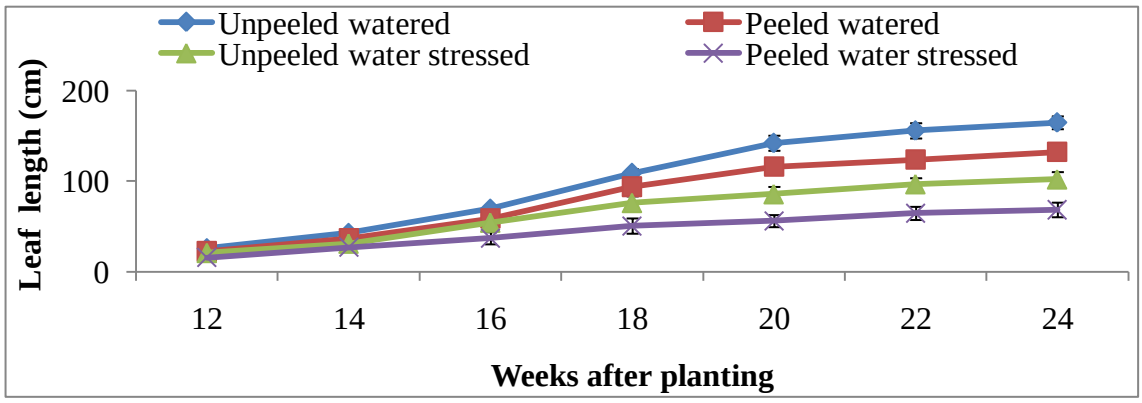
4.2.3.1 Hyacinth

In the previous sections, responses of hyacinth and lilies to herbivory have been investigated and reported. In this and the ensuing section, the combined influence of scale removal and drought, or the single influence of water stress on the two species of flowering bulbs will be discussed. The scales of flowering bulbs are not only storage sites for reserved food substances but also sites for storing water. Therefore part of the idea of scale removal in this experiment was to limit water availability to the developing plants. In the case of hyacinth, the study was carried out between November 2007 and May 2008. Bulbs of hyacinth were subjected to partial (50 %) peeling prior to planting as in case of the previous experiments but in addition, plants from this peeling regime, and those of the unpeeled bulbs (control) were either watered throughout the season or drought stressed from 4 to 24 weeks after planting. During this period, variations in weather parameters in the greenhouse were as indicated in Figure 4.29. Responses of hyacinth to partial scale removal in this experiment were a confirmation of those already mentioned under section 4.2.1 and will not be repeated. Results indicated that the plants produced from peeled bulbs had poor vigour rating (Fig 4.26c), and reduced photosynthetic rate and stomatal conductance as compared to the control (Fig 4.27a and b). Also, the plants produced from unpeeled bulbs but drought stressed recorded reductions in leaf growth (Fig 4.25a, b and c). Thus at 24 WAP, the total leaf area values were 391.46 cm^2, 210.06 cm^2 and 109.84 cm^2, respectively, for plants produced from unpeeled bulbs but watered throughout the season, plants obtained from unpeeled bulbs but water stressed, and those from peeled bulbs but water stressed. This means that subjecting the hyacinth plants to water stress alone reduced leaf growth by 46.34 % whilst the combined influence of drought and peeling reduced their growth by 71.94 %. In that same manner, unpeeled but drought stressed plants and plants from peeled bulbs that were subjected to drought suffered similar reductions in chlorophyll content (Fig 4.26b), photosynthetic rate and stomatal conductance (Fig 4.27a and b), vigour rating (Fig 4.26c), inflorescence height (Fig 4.26a) and fresh weight gain at harvest (Fig 4.28 b). Also, peeling reduced the number of florets whilst water stress did not (Fig 4.28a).

(a)



(b)



(c)

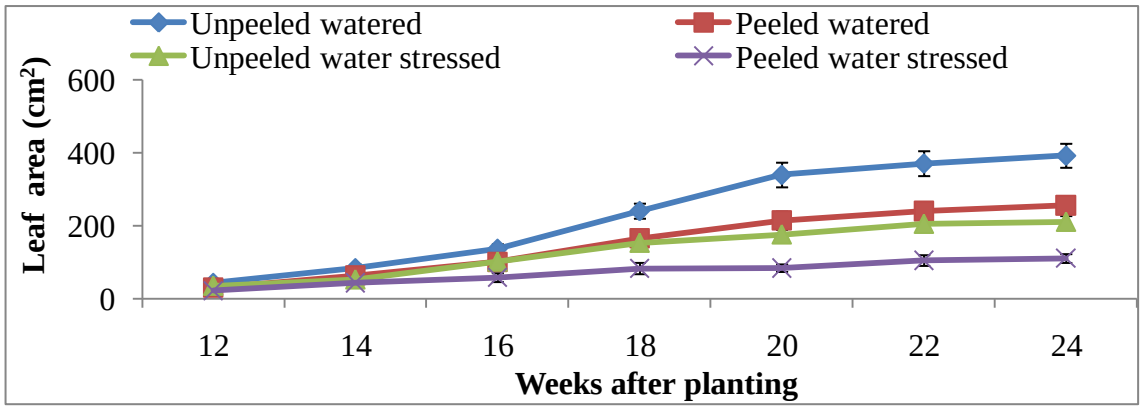
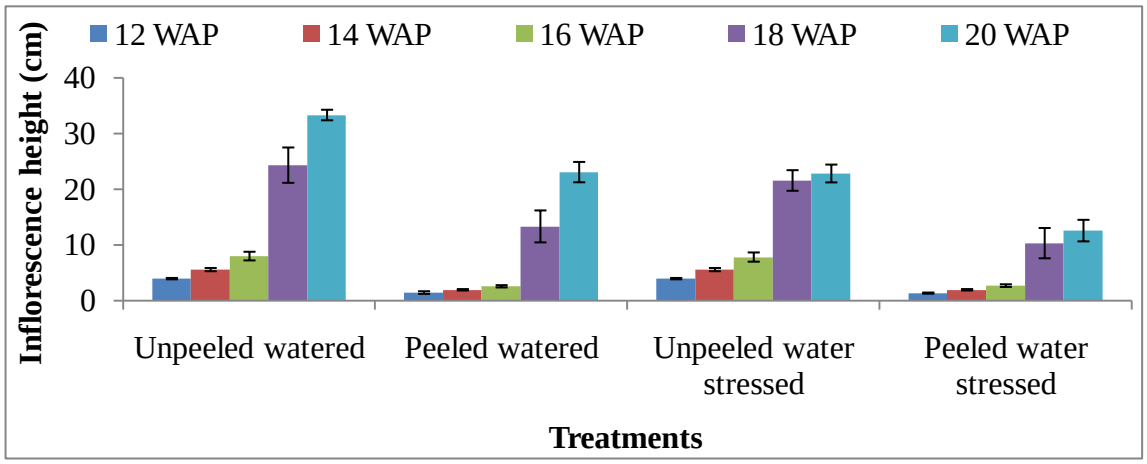
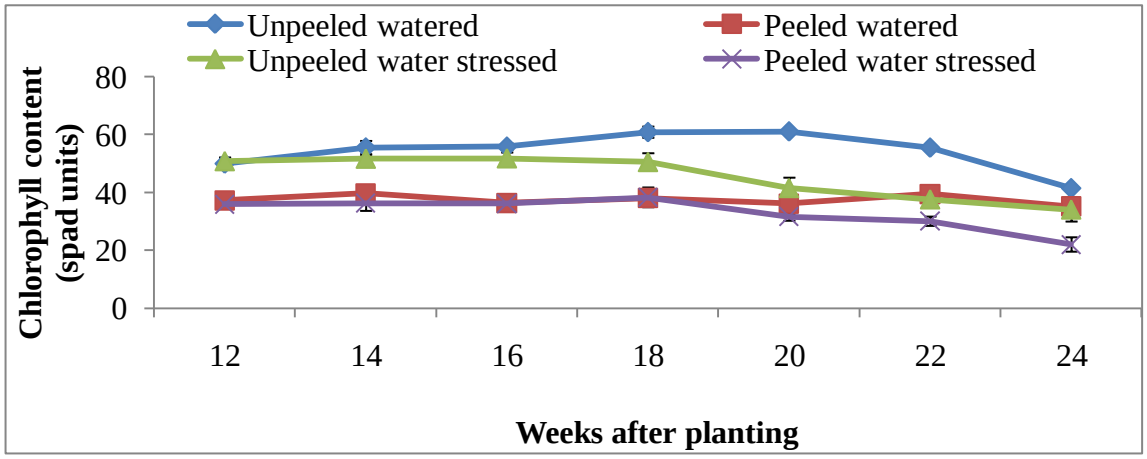


Fig 4.25: Effects of drought stress and scale removal on hyacinth leaf growth during the period of November 2007 to May 2008: (a) leaf width, (b) leaf length and (c) leaf area of the. Plants were subjected to drought at 4 WAP. Error bars represent the means ± SE of four replicates.

(a)



(b)



(c)

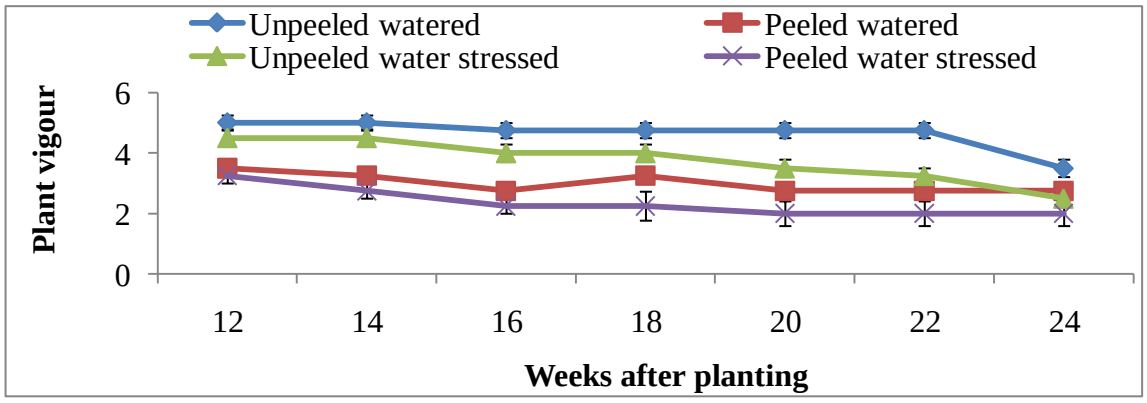
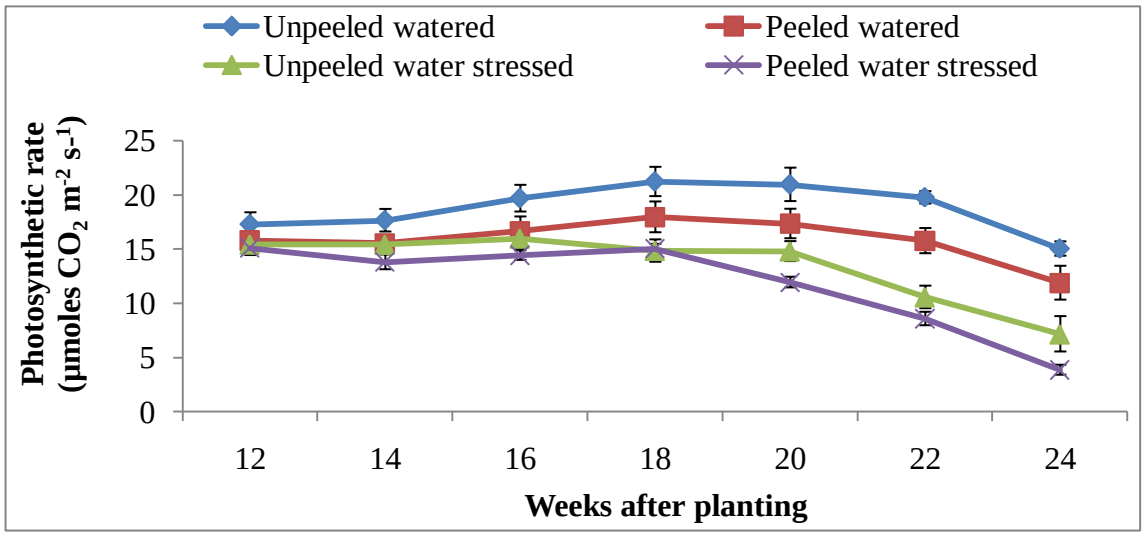


Fig 4.26: Effects of drought stress and scale removal on (a) Inflorescence height, (b) chlorophyll content, and (c) vigour of the common hyacinth during the period of November 2007 to May 2008. Vigour assessment was made by scoring the overall vigour or health of the plant stand (height, chlorophyll development, weakness, wilting etc) using a scale of 1 = very weak, 2 = weak, 3 = moderately healthy, 4 = healthy and 5 = very healthy. Data on scoring were log transformed prior to ANOVA but were back transformed for easy comparison among treatment means. Bars represent the means ± SE of four replicates.

(a)



(b)

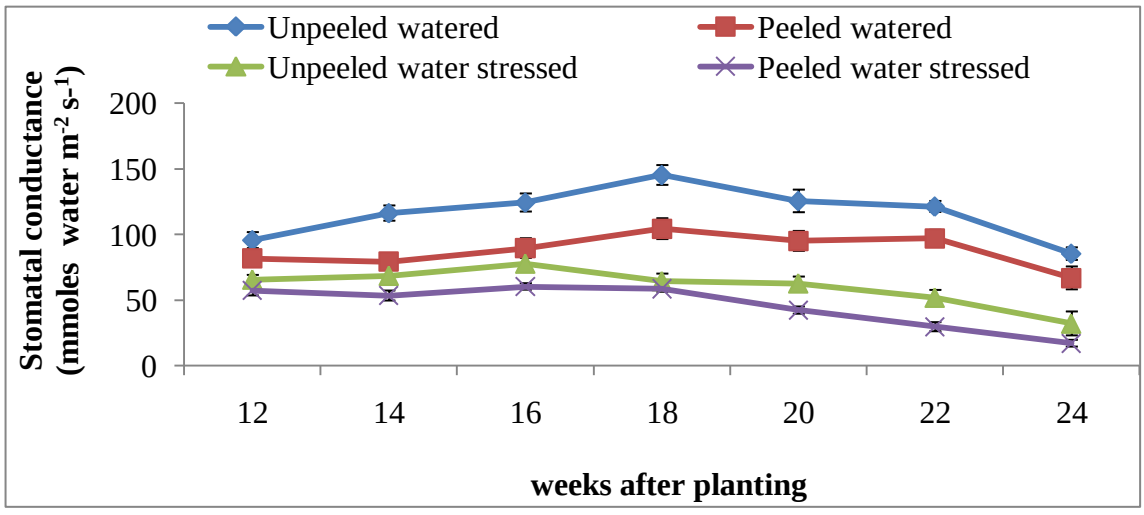
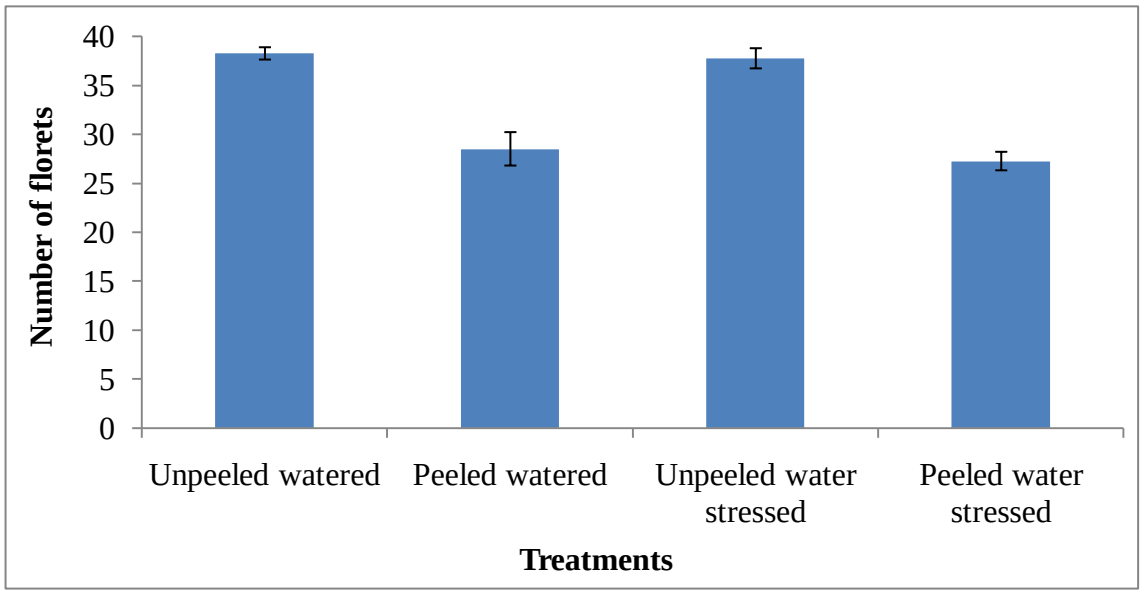


Fig 4.27: Effects of drought stress and scale removal on (a) photosynthetic rate, (b) stomatal conductance of the common hyacinth. Bars represent the means ± SE of four replicates.

(a)



(b)

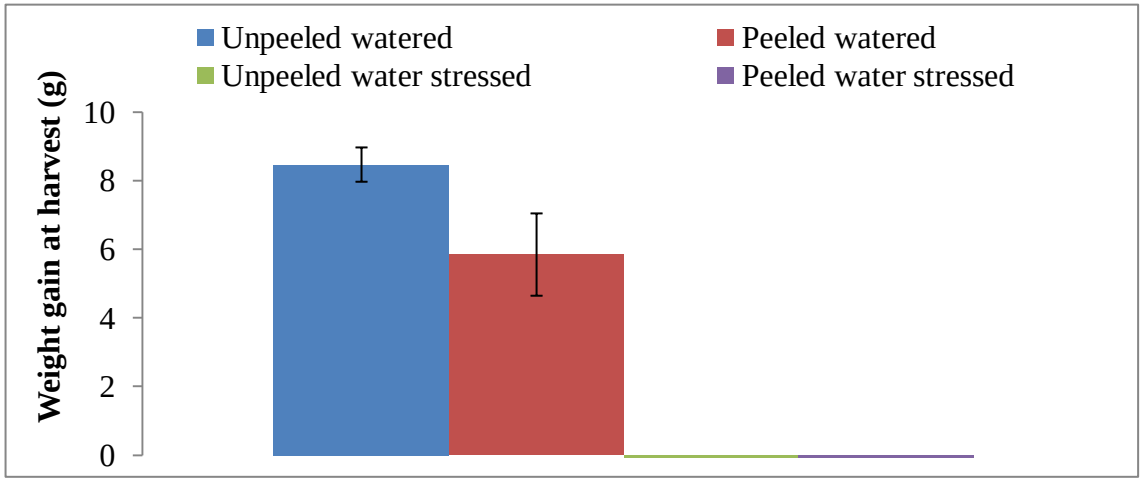
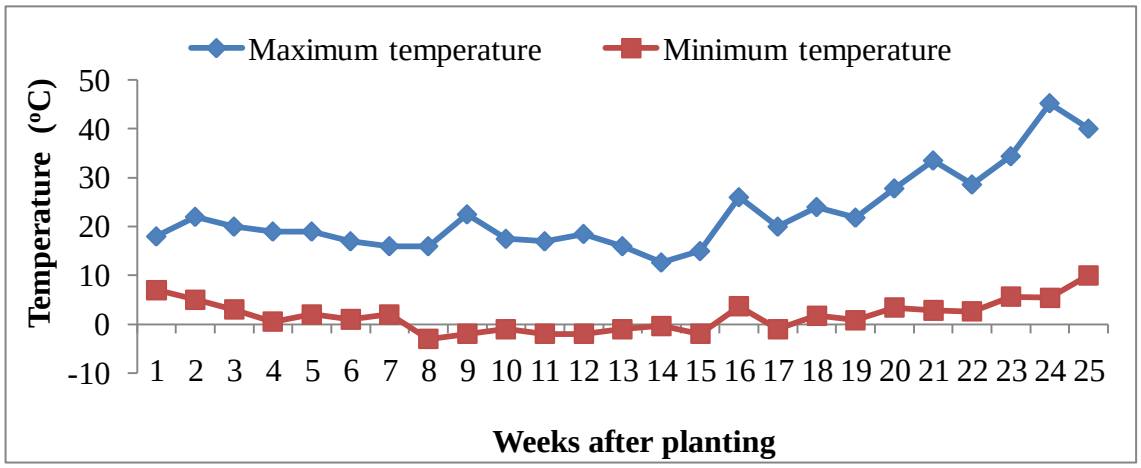
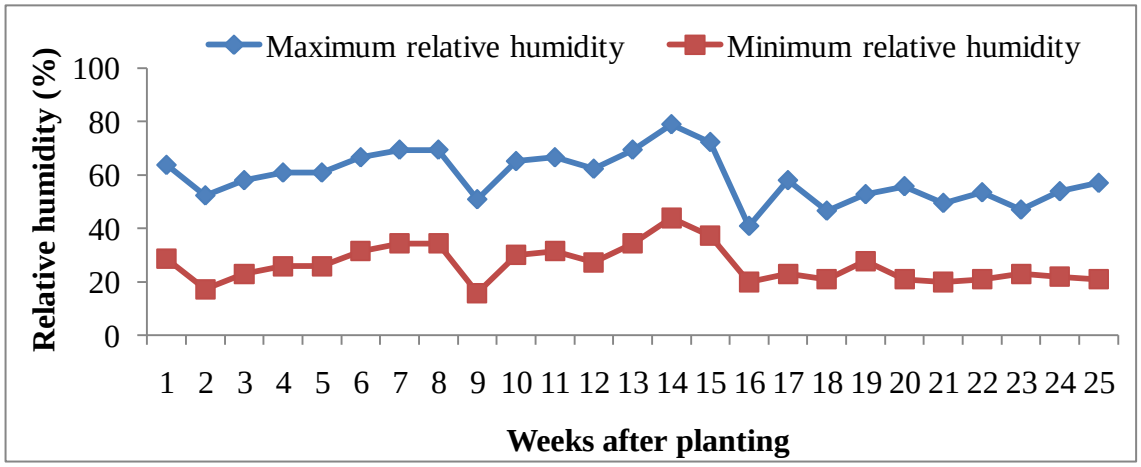


Fig 4.28: Effects of drought stress and scale removal on (a) number of florets, and (b) weight gain at harvest of the common hyacinth. Bars represent the means ± SE of four replicates.

(a)



(b)



(c)

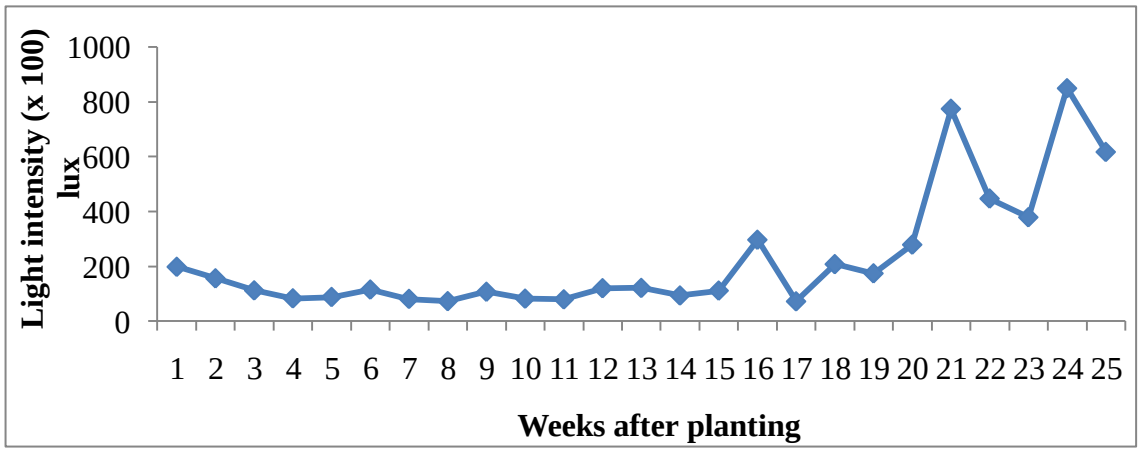
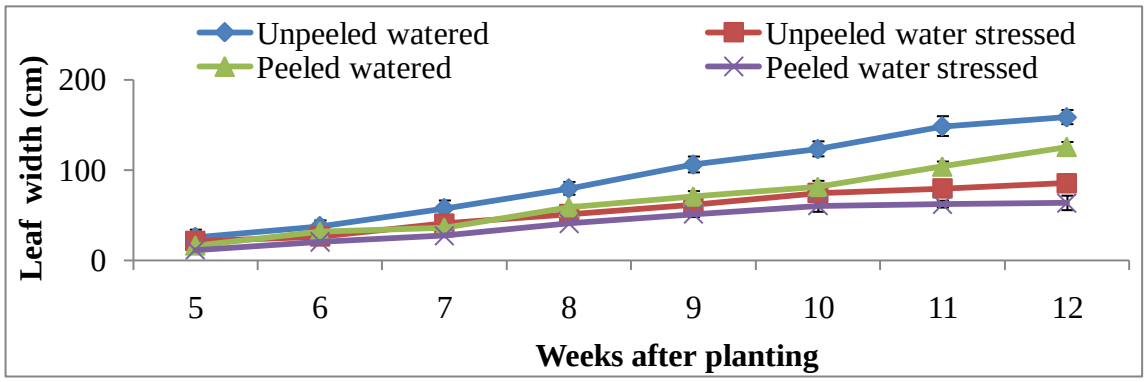


Fig 4.29: Changes in greenhouse temperature, relative humidity and light intensity during the experimental period of November 2007 to May 2008. Each value represents a mean of one week of seven days measurement, and measurements were made each day between the hours of 12.00 and 14.00 GMT.

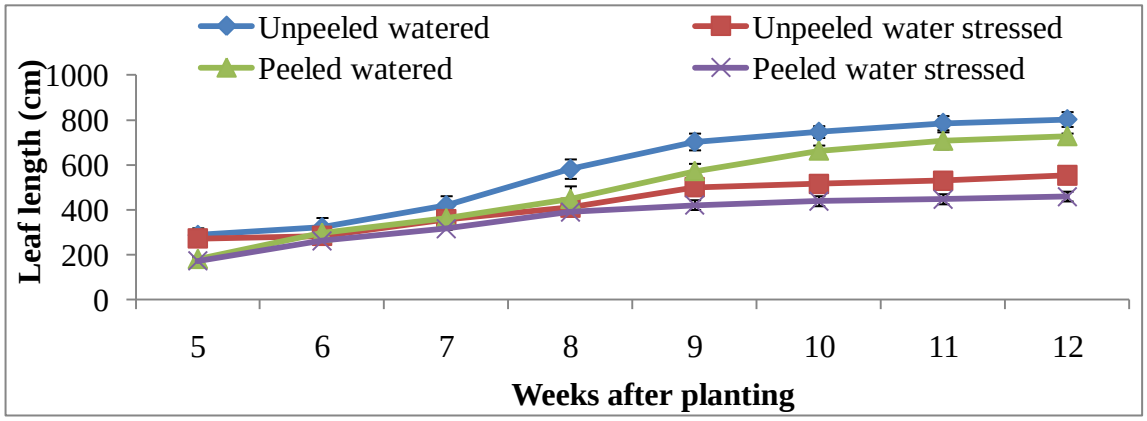
4.2.3.2 The lily

From April to August 2008, lily bulbs were also subjected to partial scale removal and drought stress, in just the same manner as the hyacinth experiment. The idea was to compare the performances of hyacinth to those of the lily in their responses to the combined influence of herbivory and drought stress but in the case of the lily experiment, water application was withheld at 3 weeks after planting. The lily bulb exhibited similar responses as those of hyacinth to peeling and drought stress (Fig 4.30 – 4.32).

(a)



(b)



(c)

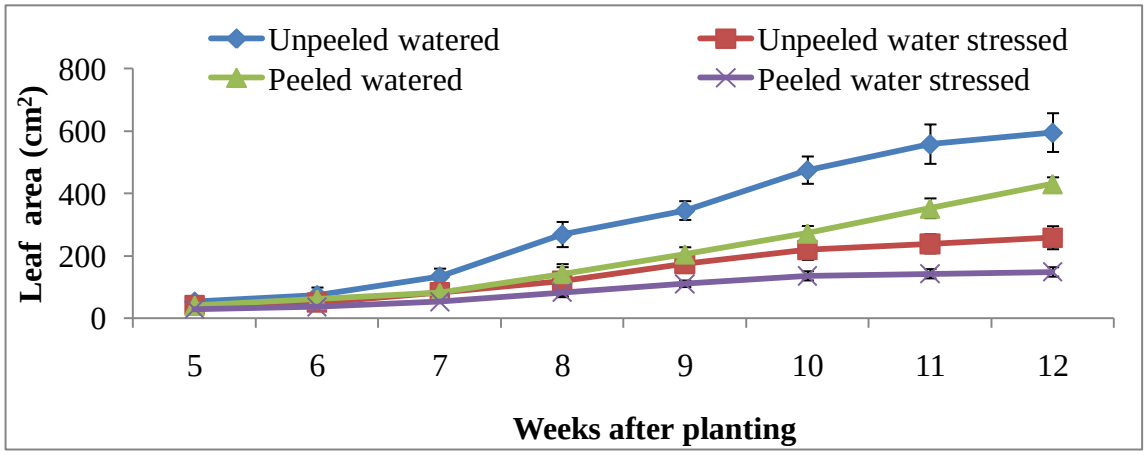
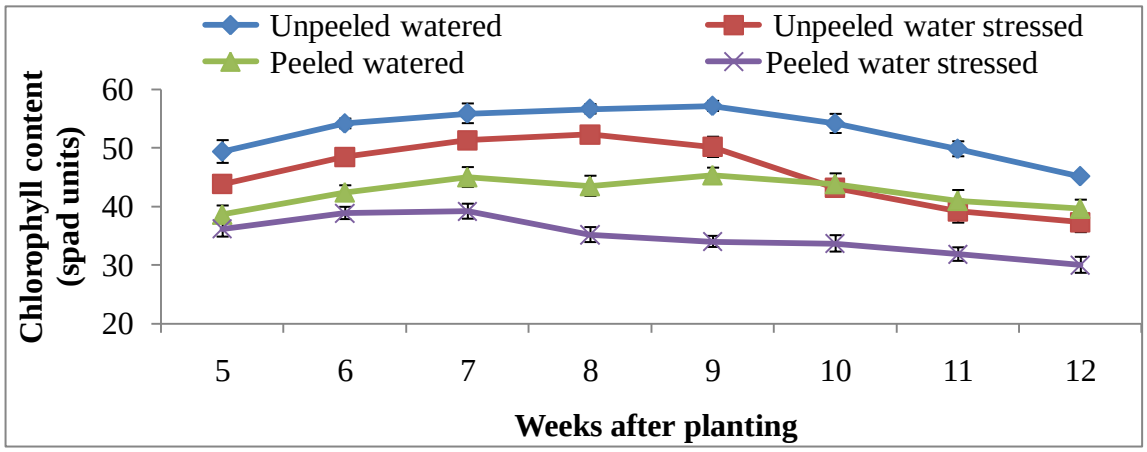


Fig 4.30: Effects of drought stress and scale removal on leaf growth of the lily during the period of April–August 2008: (a) leaf width, (b) leaf length and (c) leaf area. Drought stress started after 3 WAP. Error bars represent the means ± SE of four replicates.

(a)



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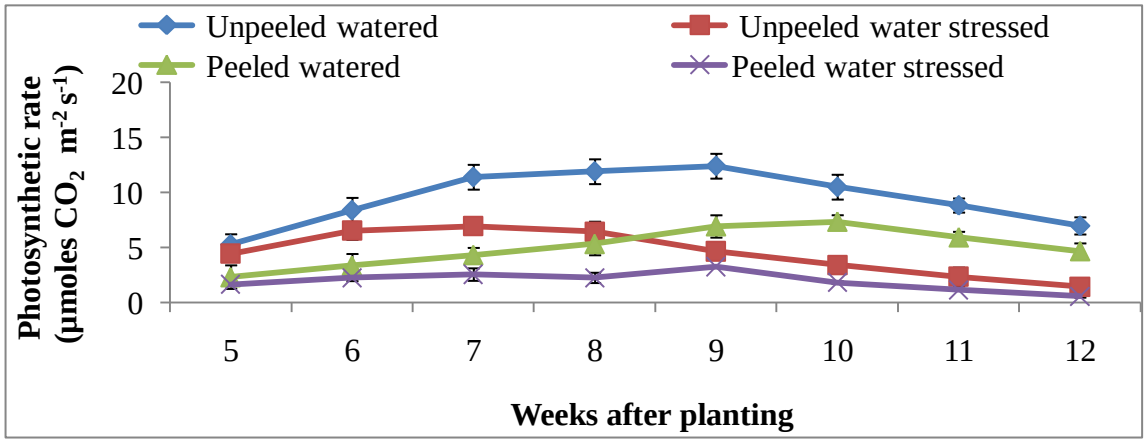


(c)

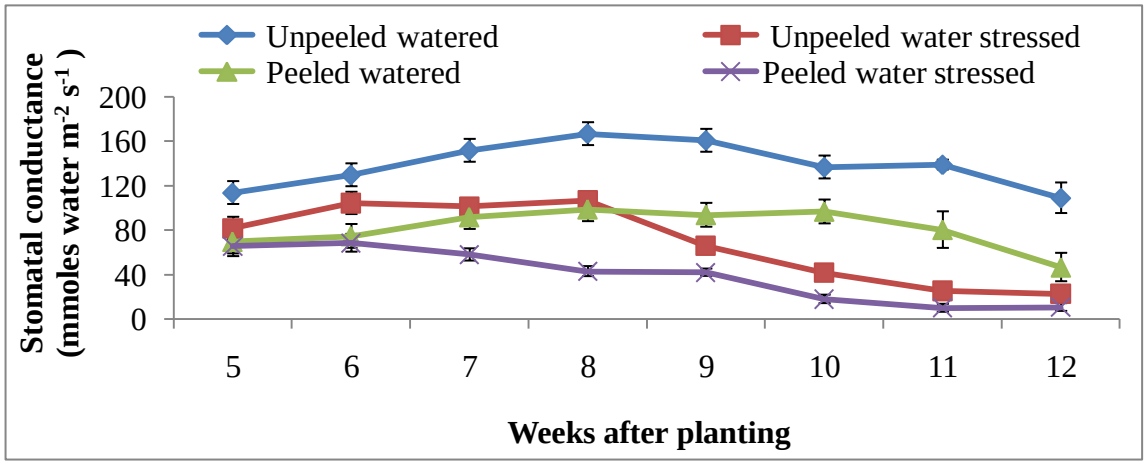
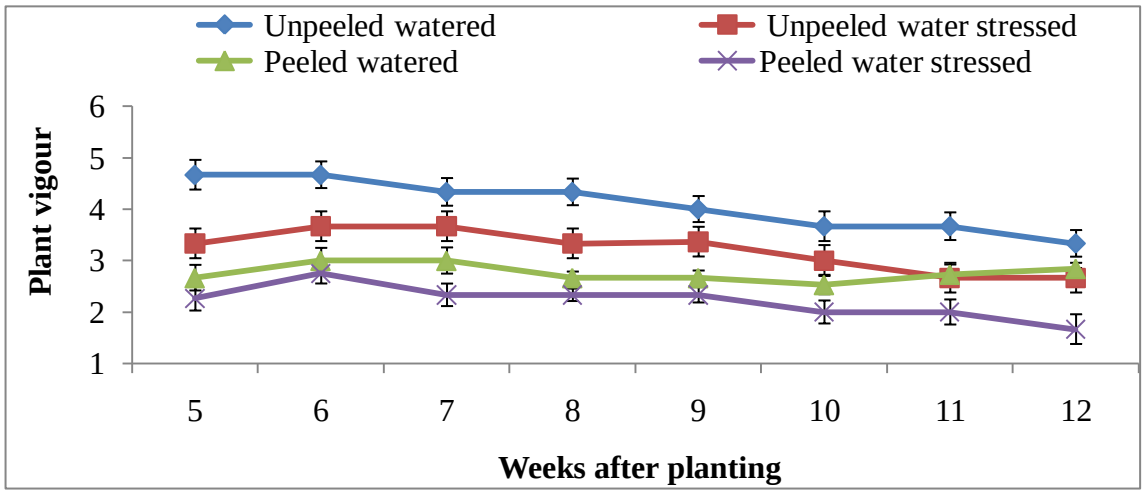
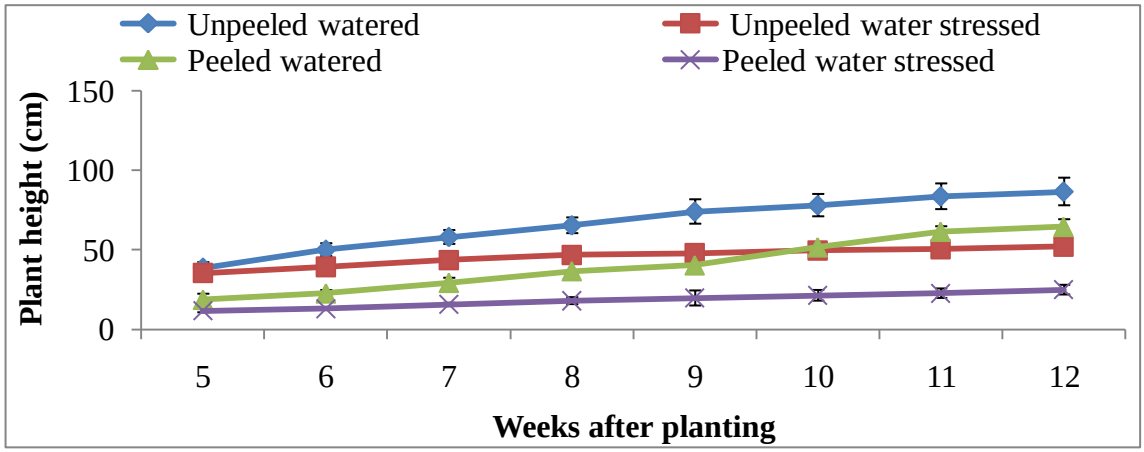


Fig 4.31: Effects of drought stress and scale removal on (a) chlorophyll content, (b) photosynthetic rate, and (c) stomatal conductance of the lily during the period April–August 2008. Bars represent the means ± SE of four replicates

(a)



(b)



(c)

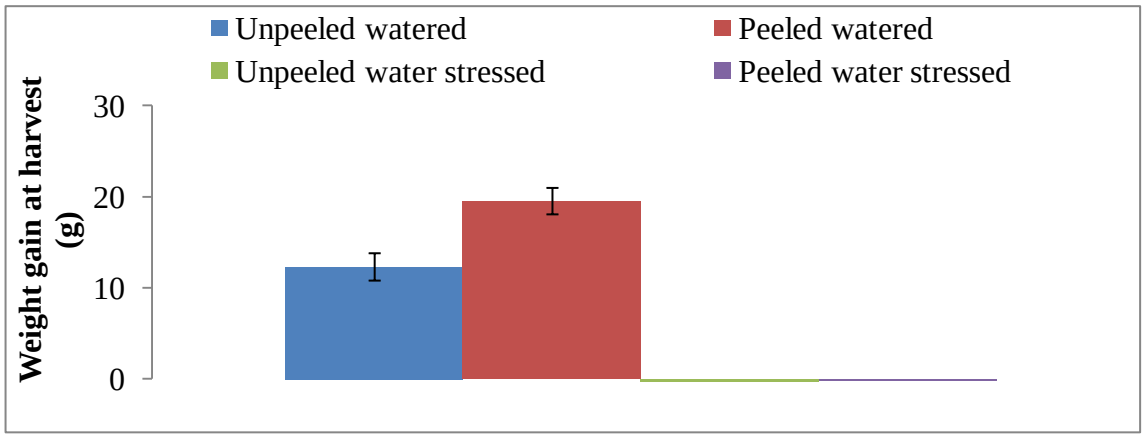


Fig 4.32: Effects of drought stress and scale removal on (a) plant vigour, (b) plant height, and (c) weight gain of the lily bulb at harvest during the period April–August 2008. Vigour was assessed in the same way as that of hyacinth. Bars represent the means ± SE of four replicates.

4.3 Discussion

4.3.1 Scale removal effects

The delay in sprouting of hyacinth bulbs that were partially peeled prior to planting during the 2006/2007 season among other factors is attributable to a reduction in food reserves and energy of the peeled bulbs. Chapter 3 has already established that, after planting of the bulbs, changes in the dry weight of the scales closely followed that of the reserve carbohydrates particularly starch, and the period of rapid reduction in starch coincided with the time of sprouting of the bulbs. This means that following planting of flower bulbs, the newly developed structures made use of the energy stored in the bulbs for emergence and subsequent growth. The removal of scales as a result of peeling prior to planting, denied the developing plants of food reserves that were necessary (reserved food serves as sources of energy) for early sprouting (Orthen, 2001; Kim *et al.*, 2003; Theron and Jacobs, 1996; Ruiz *et al*., (2002). The reduction in chlorophyll content of plants produced from peeled bulbs as compared to those from the unpeeled control also suggests that in these plants (flower bulbs), the bulb is not only a storage site for reserved carbohydrates (food reserves) or water, but in bulbous plants, the bulb is also a site for components of chlorophyll synthesis. In fact, the chemical structure of chlorophyll molecule shows that, the pigment has a chlorin ring, and at the centre of the chlorin ring is Mg^{2+} ion. This means that bulbs store minerals such as Mg^{2+} ions which are needed for chlorophyll synthesis. Thus the removal of the bulb scales as a result of peeling implies denying the developing plants of these ions that are needed for the synthesis of chlorophyll. Complete scale removal, especially when the damage occurred before planting of the bulbs, reduced the concentration of these ions drastically and this reflected not only in their chlorophyll formation, but also their photosynthetic rates. That is, complete bulb scales removal resulted in a higher reduction of ions such as Mg^{2+} in the bulb than did the partial scale removal, and this explains why plants produced from the former recorded lower values of chlorophyll concentration and photosynthetic rate than plants produced from the latter. In general, the chlorophyll content of plants whose bulb scales were peeled at two weeks after planting during the 2008/2009 season was higher than plants whose bulb scales were peeled prior to planting. Plants produced from peeling of bulbs at two weeks after planting had developed root system for water and minerals uptake before the damage occurred. But plants whose bulb scales were removed prior to planting did not have the opportunity of

developing root systems for water and minerals uptake from the soil before the damage occurred. It therefore means that, the latter were at disadvantage in terms of water and minerals absorption (resources for growth) as compared to the former. In fact, peeling of the bulb scales prior to planting delayed the emergence of the bulbs from the soil (Fig 4.02a). This means that peeling prior to planting delayed root formation and plants from this regime were at a competitive disadvantage in terms of water and minerals uptake, especially at the early stages of their development, and this adversely affected their synthesis of chlorophyll, photosynthetic rates and productivity. This observation also implies that in plants, the responses to herbivory depend on the time of herbivory during the life cycle of the plants. It also means that the stage of development of plants also influences the response of plants to herbivory. Just like the case of chlorophyll development, bulb scales removal prior to planting, or at two weeks after planting resulted in reduced vegetative growth, poor inflorescence development, and reduction in mother bulb fresh weight at harvest. The reduction in these parameters as a result of peeling suggests that not only the mother-bulb food reserves was interfered with, but also the general photosynthetic apparatus was affected, resulting in reduced photosynthetic rates, with a concomitant effects on dry matter production and partitioning of their organs. In general, the greater the severity of scale removal, the higher the interference with the mother-bulb food reserves and the damage caused to the photosynthetic apparatus of the plant. Thus, plants produced from complete bulb scale removal experienced a higher reduction in rates of photosynthesis and all other parameters measured as compared to those plants whose bulb scales were only partially peeled. Other authors such as Lee *et al.*, (2001), Kim *et al.* (2003), Rees (1971) and Rockwood and Lobstein (1994) also reported similar adverse effects of below-ground herbivory on plant growth and development. But surprising, the lily plants whose bulb scales were completely removed prior to planting produced a higher bulb yield (bulb fresh weight gain at harvest, see Fig 4.13c, Fig 4.32c, and Table 4.1) than the unpeeled control. Probably, the absence of inflorescence formation (flowers are major sink organs), might have enhanced the little assimilates produced from current photosynthesis, to be channelled to, and for the development of the bulb, instead of flower production. But in hyacinth, plants from the unpeeled control produced the highest bulb fresh weight at harvest. Differences between these two species of flower bulbs with regard to bulb yield at harvest (after subjecting them to complete bulb scales

removal prior to planting) among other factors, might be as a result of differences in their genetic make up, or it could be due to genotype x environment interaction.

It is also important to mention that since complete bulb scale removal entirely inhibited inflorescence formation in any of the species, and also partial bulb scale removal reduced flower quality (inflorescence height, inflorescence length and number of florets) as compared to those of the control, one cannot underestimate the importance of bulb scales and reserve carbohydrates to flowering and inflorescence development in flower bulbs. Flowering in bulbs appears to be intimately linked to the reserves of the planted bulb, rather than the leaf growth produced. Immature or small bulbs display no flowering until a specific bulb size is attained. The data presented herein shows that traumatic bulb damage during dormancy will lead to abortion of flowers and the bulb devoting its growth to establishing flowering sized bulb.

In 2006/2007 season, peeling of bulbs prior to planting produced plants with higher vegetative growth than did peeling at two or three months after planting. However, in year 2008/2009, it was plants produced from hyacinth bulbs that were peeled at two weeks after planting, which recorded higher vegetative growth as compared to plants whose scales were removed before planting. This demonstrates that, the response of plants to herbivory is also related to the season or the environment in which herbivory occurs. Variations in the greenhouse weather conditions may account for, at least in part, the observed differences in response of the plants (hyacinth plants whose bulb scales were removed prior to planting) to herbivory for the two seasons. Leaf growth in 2006/2007 was in general, higher than that of 2008/2009. During the 2006/2007 season, the total leaf area at 21 weeks after planting in the case of plants produced from the control was 512.4 cm^2; whilst the leaf area value of plants produced from bulbs peeled (50 % scale removal) prior to planting was 416.9 cm^2, the latter representing 81.36 % of that of the former. However, during the 2008/2009 season, the control recorded a total leaf area value of 401.71 cm^2 whilst plants from the 50 % peeling regime had a total leaf area of 132.19 cm^2 at 21 WAP, representing 33 % of those of the control. The environmental conditions that influence growth, i.e. the greenhouse temperature, relative humidity and light intensity during experimentation especially at the time of data collection were not the same for the two seasons. For instance, at the time of data collection in 2006/2007, the maximum and minimum temperature in the greenhouse varied from 16.06 to 30.93 and 1.0 to 7.0^oC respectively whilst the maximum and

minimum relative humidity also reduced from 61.99 to 44.66 % and 31.55 to 19.01 %, respectively. At the same time, light intensity ranged from 121.4 to 714.9 x 100 lux. However, during the 2008/2009 season, the maximum and minimum temperatures ranged from 16 to 32^{o}C and -4 to 9.9^{o}C whilst maximum and minimum relative humidity varied from 63.9 to 73 % and 23 to 47 %, respectively and light intensity during this season increased from 24 to 650 x 100 lux. Plants generally interact with the changes in their environment to produce a response, and the variations in seasons may influence plants' growth and development. Therefore, the differences in the greenhouse weather conditions of the two planting seasons, among other factors are responsible for the variations in the hyacinth's growth and development for the two seasons. The 2008/2009 season was not only colder and more humid, but also it was characterised by lower light intensity than that of 2006/2007; and because light must have been a limiting factor to hyacinth's photosynthetic rate in 2008/2009, the plants' vegetative growth in this season was lower as compared to the 2006/2007 season. This agrees with the observation made by Gadd, Young and Palmer (2001). According to their research, when limiting resources are more available, plants are more likely to show compensation for tissues lost during herbivory than when the resources are not available. McNaughton (1983) and also Rosenthal and Kotanen (1994) reported that the timing, and also the availability of resources in the environment to support growth, influence in one way or the other plant's responses to herbivory.

4.3.2 Above-ground herbivory

In hyacinth, plants whose leaves were defoliated did not compensate for leaf loss because growth was always reduced, but flowering was unaffected. Thus once leaves are above ground, any minor or devastating damage to the leaves will not stop the plants from flowering. The plants will continue to flower as they would have done without the damage. This contrasts with scale leaf damage of this flower bulb. Thus, even though, complete and partial defoliation in hyacinth were both detrimental to the growth and subsequent development of the bulbs, plants from the former suffered more reductions in parameters measured than those from the latter. Plants from defoliated regimes did not only experience reductions in vegetative growth but also they produced bulbs with poor fresh weight at harvest as compared to the control. The defoliated plants experienced a reduction in the size of the leaf canopy and this must have decreased the

production of photoassimilates. In general, carbohydrate reserves might have decreased following defoliation due to respiration and regrowth (Carlson, 1966). There could have been a reduction in root growth, and the production of leaves occurred at the expense of the root system. Therefore, the observed reduction in growth and bulb yield of defoliated plants may be due to the fact that these plants were placed at a competitive disadvantage relative to their control counterparts in terms of their ability to acquire resources for growth (Caldwell *et al.*, 1987; Louda *et al.*, 1990). Also, food reserves might have been used by plants that suffered these treatments for vegetative growth instead of the reserves being used to enhance productivity and these reserves might have been depleted as a result of refoliation. Rockwood and Lobstein (1994) also reported that during regrowth after defoliation, energy is invested in refoliation and is unavailable for growth and maintenance.

However, in the case of the lily, plants can respond to complete herbivory if the damage was exerted at the beginning of their growth (Fig 4.20a, 4.20b, Table 4.1). That is, lily plants that were subjected to 100 % shoot removal at 3 WAP (1st 100 % shoot removal) responded to shoot losses by exhibiting compensatory regrowth of leaves. It is possible that a number of physiological adjustments must have taken place in these plants in order to overcome the immediate loss of the photosynthetic tissues. This is because the lily plants that were subjected to complete shoot removal at the beginning of their growth produced unusually long leaves (see picture in Fig 4.20b) whose total leaf area were similar to that of the undefoliated control. In addition, the lily plants subjected to complete shoot herbivory at 3 WAP (1st 100 % shoot removal) had higher values of chlorophyll concentration, photosynthetic rate and stomatal conductance than their control counterparts. Apart from the increased photosynthetic ability of these plants, it is possible that there was a reallocation of food reserves for the production of new but longer leaves and this reflected in their high total leaf area being comparable to that of the control. These plants had no option but to depend entirely on the mobilisation of stored reserves for regrowth of vegetation and later on current photosynthesis once new leaves were developed. This agrees with the observation that plants sometimes respond to high herbivory levels with a high regrowth as a tolerant mechanism to maintain fitness (Ruiz *et al.*, 2002). In general, both hyacinth and the lily plants from the 1st herbivory regimes did not only record higher vegetative growth than their counterparts from the 2nd herbivory regime, but the former also recorded higher values of all other

parameters measured than the former. This also emphasizes that the response of plants to herbivory is related to the time at which herbivory occurs. The difference in time interval between the 1st and 2nd herbivory treatments in hyacinth and the lily were 4 and 2 weeks, respectively. In hyacinth, the 1st and 2nd herbivory treatments occurred at 14 and 18 WAP, respectively, whilst in the lily, shoot removal was carried out at 3 and 5 WAP, respectively, for the 1st and 2nd herbivory treatments. The difference in response to herbivory by plants from the 1st and the 2nd herbivory treatments, therefore, is attributable to the stage of growth of the plant at the time of herbivory, and this is also related to the amount of reserve carbohydrates present in the bulb scales at the time of herbivory. The previous Chapter produced evidence that the reserve carbohydrates especially starch content of the bulb scales, decreased after planting. This is because the newly developed organs utilised these reserves for their own growth. In hyacinth, the carbohydrate reserves (starch) of the scales decreased from 0 to 5 months (20 weeks) after planting (Chapter 1) whilst in the case of the lily bulb, these reserves decreased from 0 to 9 weeks after planting. At the time of the 1st herbivory treatments, the carbohydrate reserves were higher than the reserves at the time of the 2nd leaf or shoot herbivory. Thus, more resources (food reserves) were available to support growth and development during the time of the 1st herbivory than during the 2nd herbivory treatment. Moreover, as was observed with the scale removal experiment, both hyacinth and lily plants whose leaves or shoots were completely removed suffered more reductions in growth than those that were partially defoliated because the photosynthetic leaf surface (apparatus) of the former was interfered with and damaged more than the latter, again, emphasizing that the intensity of herbivory is very important in determining plants' response to that stress.

Table 4.1: Summary of differences between the common hyacinth and the lily in relation to their responses to herbivory

Hyacinth	Lily
1. Plants whose leaves were defoliated did not compensate for leaf loss because growth was always reduced.	Plants demonstrated considerable ability to compensate for leaf loss (complete herbivory) if the damage was exerted at the beginning of their growth (See Fig 4.20a and 4.20b).
2. Plants produced from the unpeeled control performed better than plants from all peeling regimes in terms of bulb yield at harvest.	Plants produced from complete (100 %) bulb scale removal prior to planting performed better than those from the other peeling regimes and the unpeeled control in terms of gain in bulb fresh weight at harvest (Fig 4.13c).

4.3.3 Drought stress and scale removal effects

Growth and productivity of hyacinth and lily was negatively affected by drought. Plants produced from unpeeled bulbs that were subjected to drought suffered a reduction in vegetative growth and chlorophyll content as compared to their counterparts that were watered throughout the season. The reduction in the photosynthetic apparatus of drought stressed plants, coupled with their poor chlorophyll formation led to a reduction in their photosynthetic rates and stomatal conductance as compared to those plants that were watered. Pelah *et al*. (1997) and also Hsiao (1973) also reported that drought stressed plants exhibited stomatal closure, low photosynthetic rates, reduced transpiration rates and growth, and also a reduction in productivity. The reduction in the essential process of photosynthesis of hyacinth or the lily plants subjected to drought was not only due to a reduction in their chlorophyll content or lowered photosynthetic apparatus, but in general, during drought stress, plants react to water loss by exhibiting a combination of adaptive traits such as increasing solute concentration in the plant environment, and this causes an osmotic flow of water out of the cells of the plant. The result is that, water potential of the plant cells decreases leading to membranes disruption, acceleration of the natural process of ageing (senescence) and poor yield. In this work, not only was chlorophyll content of plants subjected to drought reduced, but these plants also suffered early flower abortion, wilting and shoot die back which are all indications of early senescence. This is in agreement with the observation made by

Volaire (2002) on *Dactylis glomerata*. According to her research, during water stress, leaf growth of this species decreased with declining soil moisture, and the proportion of senescent tissues in total aerial biomass increased in drought stressed plants relative to the control. Garwood and Sinclair (1979), and also Volaire and Lelievre (2001) explained that plants hastened senescence of their aerial tissues during water stress so as to delay or limit the rate of dehydration or water loss and this was the case of the two bulbous species subjected to drought in this present study. Also, the reduction in stomatal conductance and photosynthetic rates of plants subjected to drought, as well as their low vigour rating as compared to those watered, led to poor bulbs yield at harvest. Alexseev (1950) and (Maximov, 1939) also observed that in plants, water deficiency is harmful because plants exposed to drought are not only small and weak, but they have reduced growth and yields. The research reported here suggests that although geophytic plants like hyacinth and lilies survive dry periods as bulbs, both plants were very susceptible to drought stress in the growing phase of their life cycle. Any adaptations bulbs possess to survive drought must be invested in the dormant bulb. The detrimental effects of bulb scale removal (peeling) prior to planting on growth and development of the bulb has already been discussed under section 4.3.1 and will not be repeated here. Thus plants produced from peeled bulbs, which were also subjected to water stress underwent double stresses i.e. the combined action of scale removal and drought stress, and these were responsible for their poorest performance in terms of all parameters considered for measurement in this work.

CHAPTER 5

SOME AGRONOMIC PRACTICES

5.1 Introduction

Flower bulbs are grown mainly for the production of cut flowers, potted plants or as landscaping plants. These plants therefore occupy a significant position within the world-wide production and trade in cut flowers (VBN Statistiekboek report, 2002). According to Heins *et al*. (2000), consumers often require detailed specifications from growers involved in horticultural flower bulb production for products they buy both in times of delivery, and in terms of quality factors such as height and flower number. This implies that growers must perform proper cultural and management procedures at the right time so as to meet the needs of consumers. Plants, however, are grown in environments whose indices vary in a number of parameters: disease and pest infestations, soil moisture availability, soil nutrient levels, soil and air temperature, light duration, light intensity and relative humidity. But in general, the growth and developmental processes of plants interact with the environmental factors to influence the level of productivity or quality of that plant. One should not, therefore, underestimate or belittle the environment in which the plant is growing. Manipulating the immediate environment of plants by embarking on sound agronomic practices will not only allow plants to grow optimally, but will enhance the production of good quality products and yield.

In general, it has been established that, the major production problems in bulb production are extended flooding during harvest times, excessive heat and drought especially during hot summer seasons, very cold winter seasons, and the occurrence of diseases and pests. Flooding is known to cause rot problems, or sometimes the complete loss of the flower bulb. Very high temperatures and drought may result in wilting which ultimately reduces bulb growth, and this may increase the number of small bulbs which are not of marketable sizes. Excessive heat and drought can also increase the incidences of pests and diseases, and during the winter season, unusually low temperatures may result in the accumulation of ice or snow on plants, resulting in breakages. In addition, sudden low temperatures sometimes lead to swelling and bursting of cells of plants, with the result that, disease pathogens might gain entry into the bulb plant to cause infections. According to Doyle *et al*. (1995), however, in plant production, most of the production problems are controlled when growers practise good management or agronomic practices. For instance, in bulb production, the use of pumps to remove water from fields which are flooded can minimize flooding and the associated rot

problems. The problem of drought and excessive heat may be solved by practising mulching to check water loss by evaporation and to conserve soil moisture, or embarking on irrigation to make water available for plant uptake. Many growers have also carried out soils testing and fumigation to control nematode infestation and other related soil-borne diseases. In addition, the use of herbicides and pesticides will check weeds and pests, respectively; and adopting husbandry practices such as the timely use of fungicides, crop rotation, use of resistant cultivars (genotypes), planting at high densities, and hot water treatments prior to planting (Hanks, 2002) have proved effective in controlling most diseases during the production of bulbs.

In plant production, the importance of supplying nutrients to correct soil infertility and to increase productivity cannot be overemphasized. Nutrients such as nitrogen, phosphorus and potassium play a major role in the growth and development of plants (Scott, 2008). These essential nutrient elements improve the chemical and biological properties of the soils, and therefore enhance higher yields of plants. Research by a number of authors: Silberbush *et al*. (2003), Kim *et al*. (1998), Clemens *et al*. (1998), Engelbrecht (2004) and Louw (1993), have all emphasized the need to supply nutrients to the soil during the growth of plants. Equally important to the growth and development of plants is the depth at which the planting material is planted. However, the optimum depth is dependent on plant type, size of planting material and the prevailing environmental conditions (Vogel, 1963 and Stickler, 1962). In bulb production, choosing the correct depth of planting is a very important decision paramount to the production of the species (Kilkelly, 2006). This is because, when bulbs are planted too shallow, it could lead to frost damage or premature emergence of shoots and sometimes frost heaving, a situation that occurs when bulbs are pushed up out of the soil by freezing temperatures. Pests such as birds and rodents can also damage bulbs that are planted too shallow. On the other hand, when bulbs are planted too deep, there can be a problem of emergence failure or production of poor quality flowers. Research on the specific details on the influence of planting depth on the growth of bulbs is lacking and this needs to be addressed. In general, the growth and yield of geophytic plants is directly proportional to the amount of food reserves present in the material used for propagation at the time of planting (Bremner and El Saeed, 1963; Burton, 1966; Rees, 1969). Hidekazu *et* al. (1998) compared different seed tuber sizes of yam on growth and yield of the crop as well as sprouting and biomass reduction

associated with the seed tuber sizes. They reported that the size of the yam seed tuber used for propagation influenced both the degradation of reserves stored in the tuber and the eventual growth and development of that plant because the characteristics of the above-ground organs of yam: stem length, number of branches, total leaf area and the length as well as the weight of the newly developed tubers positively correlated with the initial seed tuber size. Also, the reduction in biomass and reserves during sprouting was higher in the large seed tubers than in the case of the small seed tubers and this explained why there was a higher growth and yield of the former as compared with the latter. The authors therefore concluded that seed tuber size was very important in the selection of planting materials for cultivation. Rees (1985) also reported that even when conditions are favourable, geophytes which are too small in size would fail to flower; and flower quality (number of flowering shoots, number of florets per inflorescence, and stem length) often improves as the size of the planting material increases. However, in *Brodiaea*, Han *et al.* (1991) stated that, the percentage of the corm that flowered, and flower quality was independent of the mother corm size used for propagation, but rather it was the size of the apical meristem that determined the quality of the flowers produced. The importance of carbohydrate reserves of bulb scales to the initial growth (Chapter 3) and inflorescence formation in flowering bulbs has already been discussed. Similarly in Chapter 4, it was established that scale excision adversely affected inflorescence formation in either hyacinth or the lily bulb. Since bulbs are generally grown for flower production, and there is a relationship between the reserves of the bulb scales and flower production, large bulbs are expected to have higher vegetative growth and development than small bulbs because the former have a relatively higher reserves and volume of scales than the latter. However, information on the particular bulb sizes that will produce good quality flowers and bulb yield in hyacinth or the lily has not been documented in the literature, but such data is necessary because it would allow growers to select the right sizes of these species for propagation in order to achieve products which are of good quality for consumers. The period between October 2008 and November 2009 in my DPhil Studies was therefore devoted to studying the responses of bulbs of hyacinth and the lily to the following agronomic practices: depth of planting, nutrients supply, influence of bulb size at planting and the role of varieties in the growth and development of these bulbs. Specifically, this study aimed at determining which bulb size, planting depth, nutrients type and level, or variety would give the best quality either in terms of flower production, bulb yield or both.

5.2 Results

5.2.1 Influence of bulb size at planting on growth and development

5.2.1.1 Hyacinth

The previous Chapter produced evidence about peeling of bulb scales and the detrimental effects it has on growth, flower production and yield of bulbs as compared to the unpeeled control. This is because scale removal, through chopping (peeling) to reduce the sizes of the planted bulbs, reduced or completely eliminated (chopped away) the amount of reserves stored in the bulbs scales, on which the developing plant depend for their initial growth and development. This prompted the research to also investigate the influence of a range of bulb sizes, assumed to have varying levels of carbohydrates reserves, on the overall growth and development of the bulbs. Thus in hyacinth, eight bulb sizes (expressed in terms of fresh weight prior to planting) namely: 10, 20, 30, 40, 50, 60, 70 and 80 g were evaluated between October 2008 and June 2009 to determine the effects of bulb sizes at planting on growth and development of the bulb. Results showed that in general, the parameters measured increased with increasing bulb size at planting. This observation is also supported by the high correlation coefficients between bulb size (BS) and the measured parameters (Table 5.1). Leaf width and area correlated better with bulb size (r = 0.75, 0.68, respectively; Table 5.1) than leaf length (r = 0.47). Vast differences existed in leaf growth of plants obtained from the smallest and largest bulb sizes. For instance, in the case of the plants obtained from the smallest bulb size (10 g size), leaf width, length and area increased from 3.95 cm, 7.90 cm and 6.01 cm^2 at 12 weeks after planting to 10.99 cm, 132.58 cm and 214.37 cm^2, respectively, at 24 weeks after planting (Fig 5.01 a and b; Fig 5.02 a). These increases represent relative growth rates of 0.084 cm day^{-1}, 1.484 cm day^{-1} and 2.480 cm^2 day^{-1}, respectively. In the case of those from the largest bulb (80 g size), leaf width, length and area increased from 9.79 cm, 13.83 cm and 20.93 cm^2 at 12 weeks after planting to 28.13 cm, 215 cm and 689.89 cm^2, respectively, at 24 weeks after planting, giving relative growth rates of 0.219 cm day^{-1}, 2.394 cm day^{-1} and 7.964 cm^2 day^{-1} respectively. Chlorophyll content of plants obtained from the large bulbs was generally higher than those of the small bulbs (Fig 5.02 b) but the reduction in this parameter from 16 or 18 to 22 weeks after planting was higher in the case of the large bulbs than in the cases of the medium and small bulbs. For plants produced from the small bulbs (10, 20 and 30 g sizes), the leaf chlorophyll concentration generally increased from 12 to 22 weeks after planting whilst

in the case of the large bulbs (60-80 g sizes) chlorophyll content increased from 12 to 18 weeks after planting, and dropped from this point to 22 weeks after planting. The chlorophyll content of plants from the medium bulb (40-50 g) also dropped at 18 weeks after planting. Thus for example, whilst there was no reduction in chlorophyll content of plants from the small bulbs (10-30 g sizes), in the case of those from the 40 g bulb size, chlorophyll content dropped from 69.48 to 62.31 spad units from 16 to 22 weeks after planting, representing 10.31 % reduction in chlorophyll whilst in the case of those from the 70 and the 80 g bulbs, leaf chlorophyll concentration reduced from 75.14 to 51.97 spad units and from 75.75 to 58.02 spad units, respectively, and these represents 30.8 % and 23.41 % reduction in chlorophyll. Inflorescence development and characteristics (flower quality): inflorescence height, inflorescence length, inflorescence diameter and inflorescence stalk diameter all followed the distribution pattern exhibited by leaf growth, that is all these parameters increased with increasing bulb size at planting (Fig 5.03a and b; Fig 504a and b; Fig 5.05a). The rate of growth in height of the inflorescence was gradual from 14 to 18 weeks after planting but very sharp at 20 and 22 weeks after planting. Also, at 20 and 22 weeks after planting, the plants obtained from 60, 70 and 80 g bulbs recorded similar values of inflorescence height and length, (Fig 5.03a and b). Similarly, there was no significant difference in inflorescence diameter for those produced from 50, 60, 70 and 80 g bulbs at 20 and 22 weeks after planting. However, there was a vast difference in terms of inflorescence development for the plants obtained from the 10 and 80 g bulbs. For example, from 14 to 22 weeks after planting, plants from the 10 g bulb size recorded values of inflorescence height and length of 1.67 - 17.67 cm, and 1.17-3.43 cm respectively, whilst those obtained from the 80 g bulb size had 4.67 - 37.67 cm and 6.67-16.33 cm. Similarly, very few florets were observed from plants produced from the small bulbs as compared to those of the large ones. Average number of florets ranged from 2.33 in the case of those from the 10 g bulb size to 12.33 for those belonging to the 40 g bulb size, whilst in the case of plants from the large bulbs (60-80 g), number of florets ranged from 55 to 73 (Fig 5.05 a). The relationship between number of bulblets and bulb size at planting was not clear ($r = 0.39$, Table 5.1) but the fresh weight of bulblets generally increased with increasing bulb size at planting (Fig 5.05b). This is also confirmed by the high correlation coefficient ($r = 0.81$, Table 5.1) between bulb size (BS) at planting and fresh weight of bulblets (FB) at harvest, and the low correlation coefficient between bulb size at planting and number of bulblets (NoB) ($r = 0.39$, Table 5.1). Fresh weight of the mother

bulb at harvest followed the same distribution pattern as number of florets (Fig 5.06) but the percentage gain in weight at harvest was inversely related to bulb size at planting. In general, measurements made of hyacinth in this experiment revealed that, after the 50 or 60 g bulb size, values of parameters appeared to be similar in magnitude, and in most cases, differences were not significant above the 50 g bulb size. This means that, the 60 g, 70 g and the 80 g bulb sizes recorded similar values of either vegetative growth or flower quality, and the possession of large bulb size, in hyacinth, seemed to be of little advantage to the plant in terms of growth and flower production.

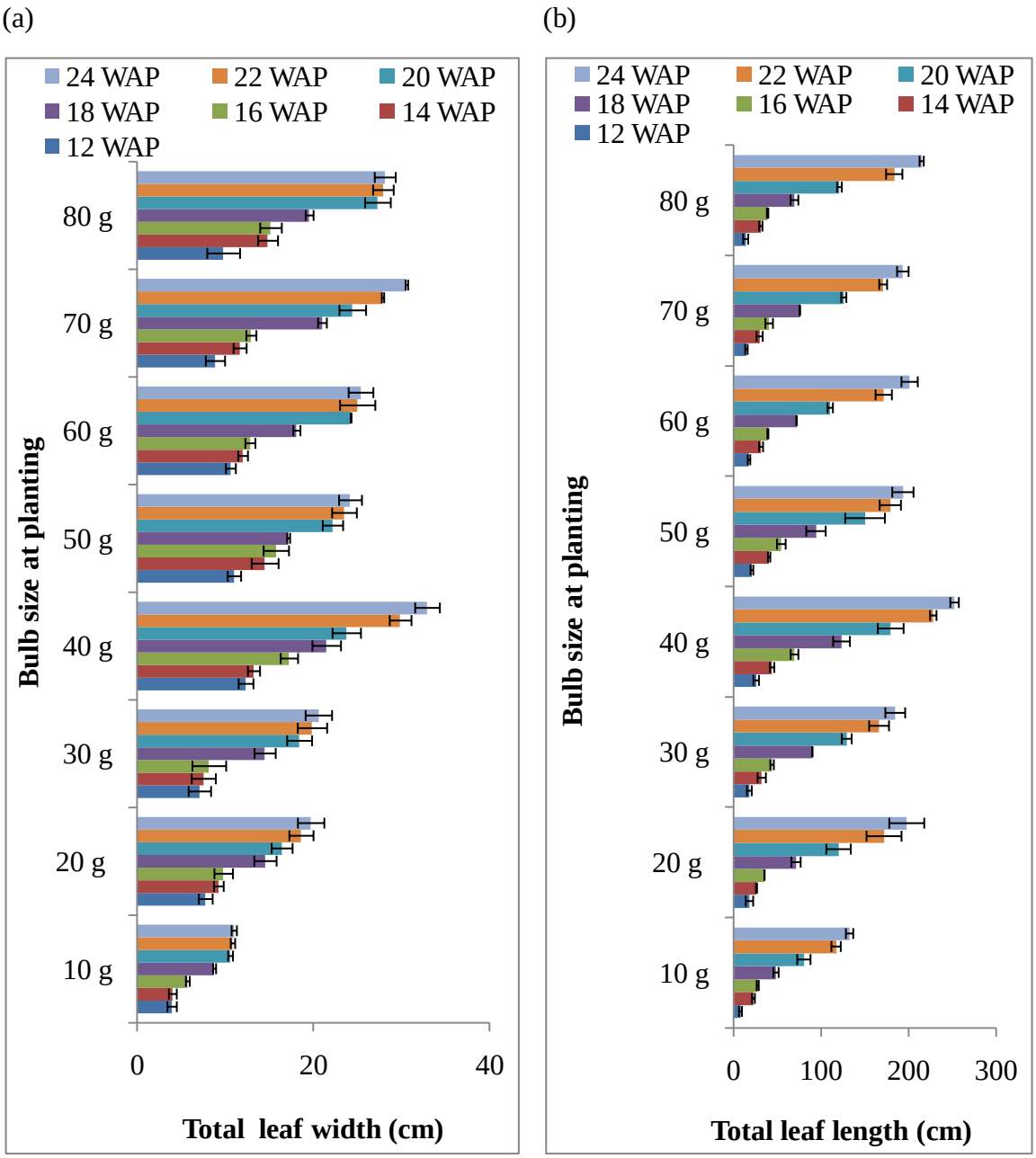


Fig 5.01: Effects of bulb size at planting on leaf growth of hyacinth: (a) total leaf width, and (b) total leaf length. Measurements were made on five plants per treatment (bulb size at planting) and the results shown are mean values ± standard errors.

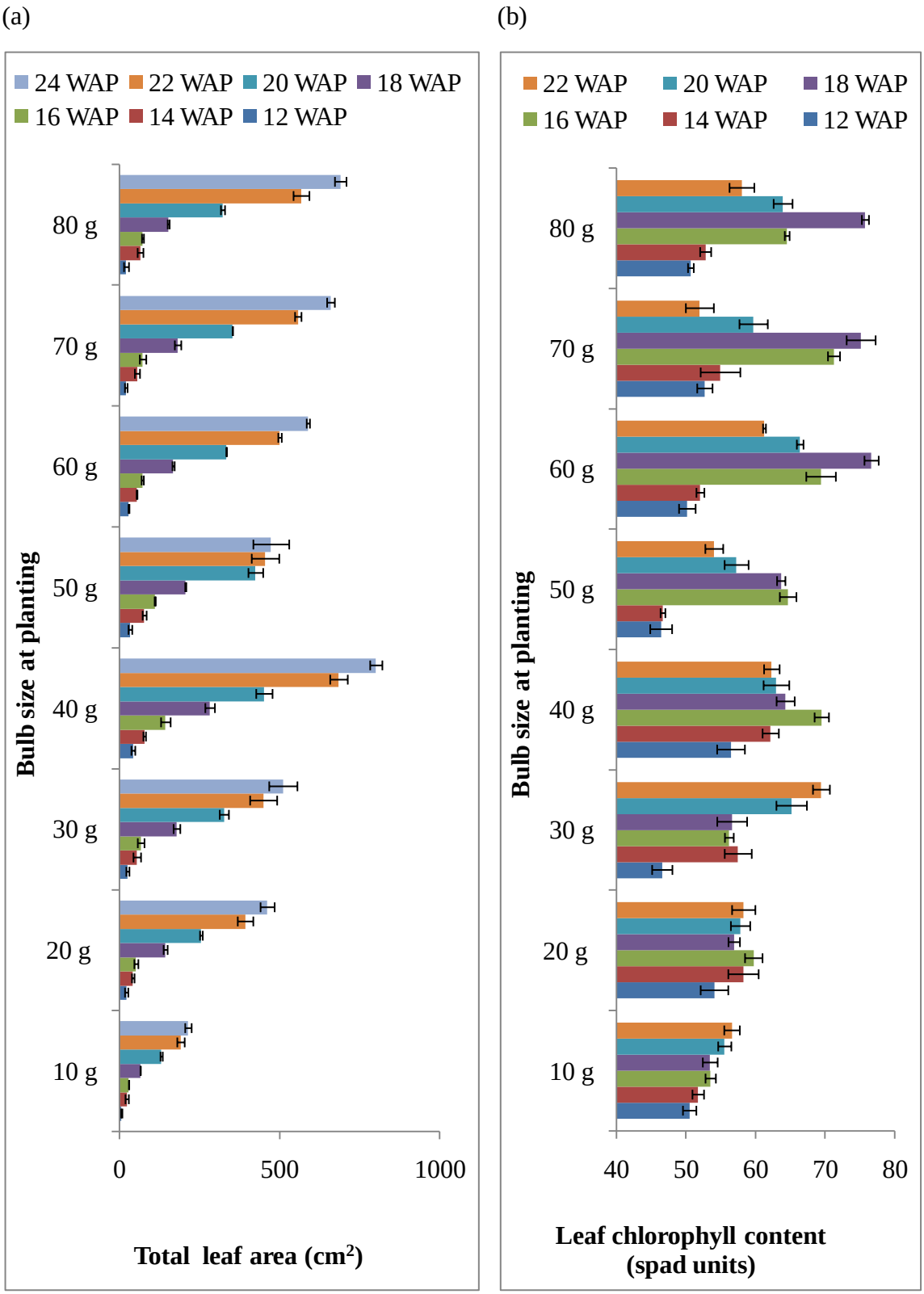


Fig 5.02: Influence of bulb size at planting on: (a) total leaf area and (b) chlorophyll content of hyacinth. Results are mean values ± standard errors of five plants per treatment.

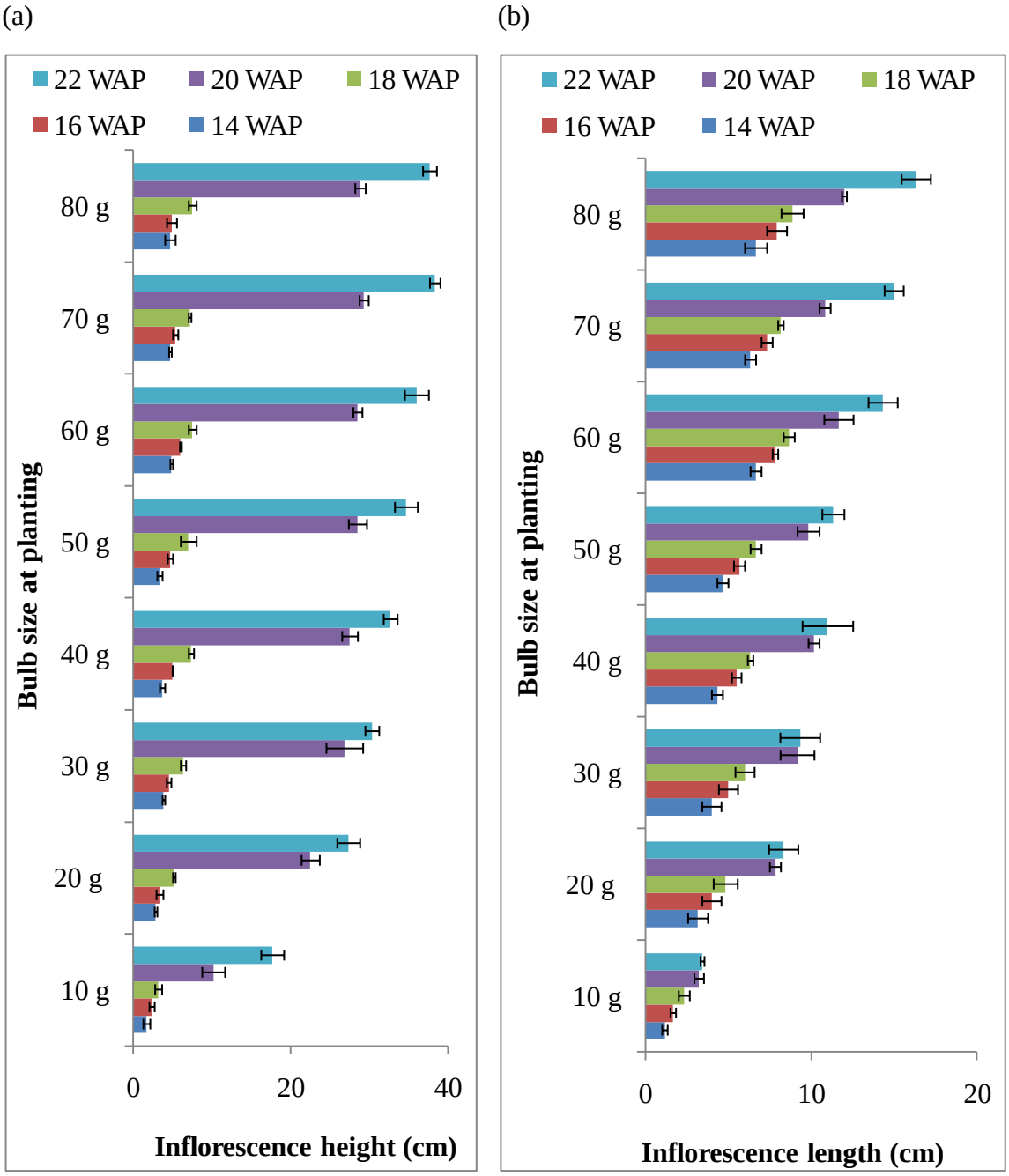


Fig 5.03: Influence of bulb size at planting on hyacinth inflorescence development: (a) inflorescence height and (b) inflorescence length. Values are means ± standard errors of five plants per bulb size.

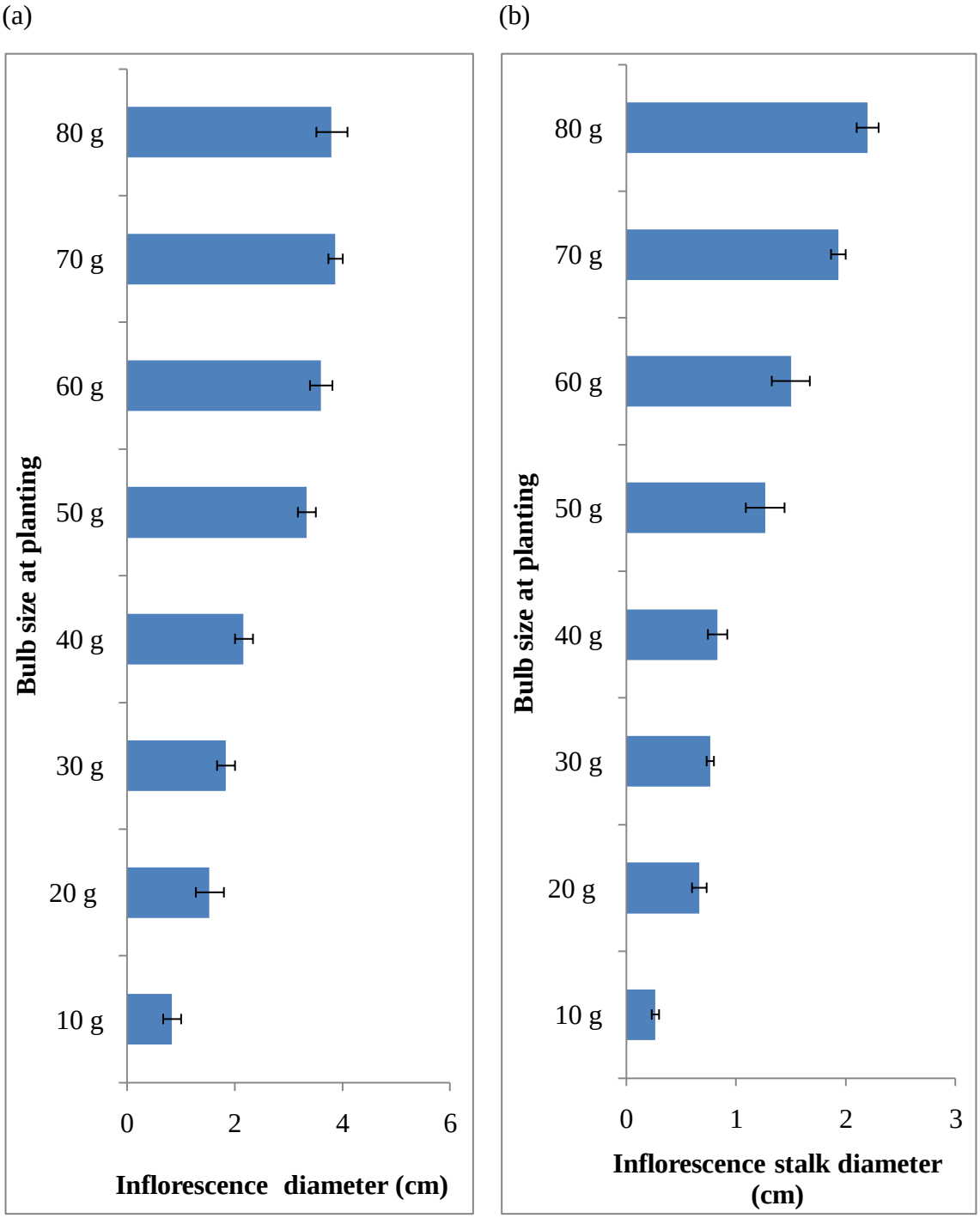


Fig 5.04: Bulb size at planting effects on: (a) inflorescence diameter and (b) inflorescence stalk diameter of hyacinth. Values shown are means ± standard errors calculated from five plants per bulb size.

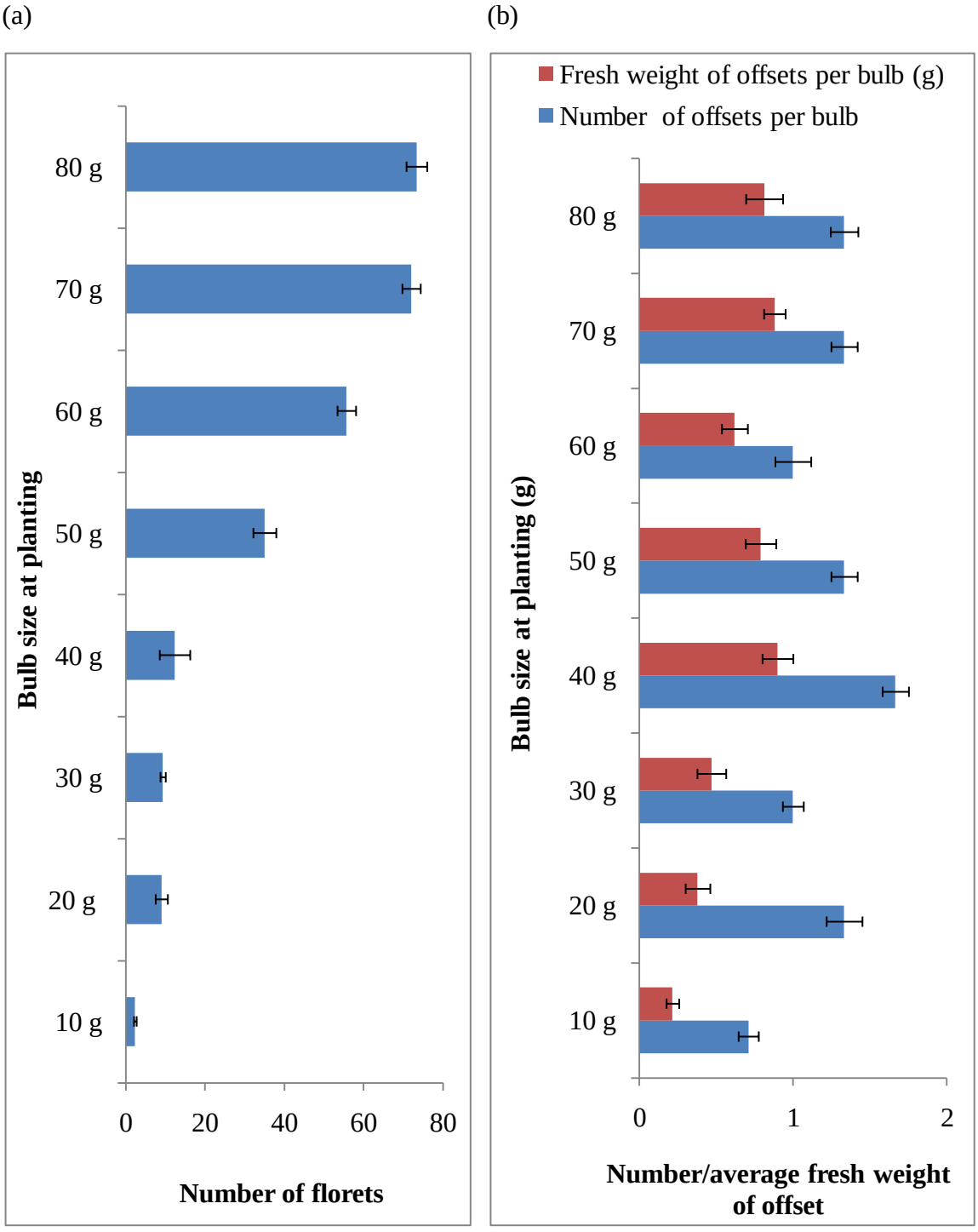


Fig 5.05: (a) Number of florets, and (b) bulblets formation in hyacinth as influenced by bulb size at planting. Results are means ± standard errors of five replicates for each bulb size.

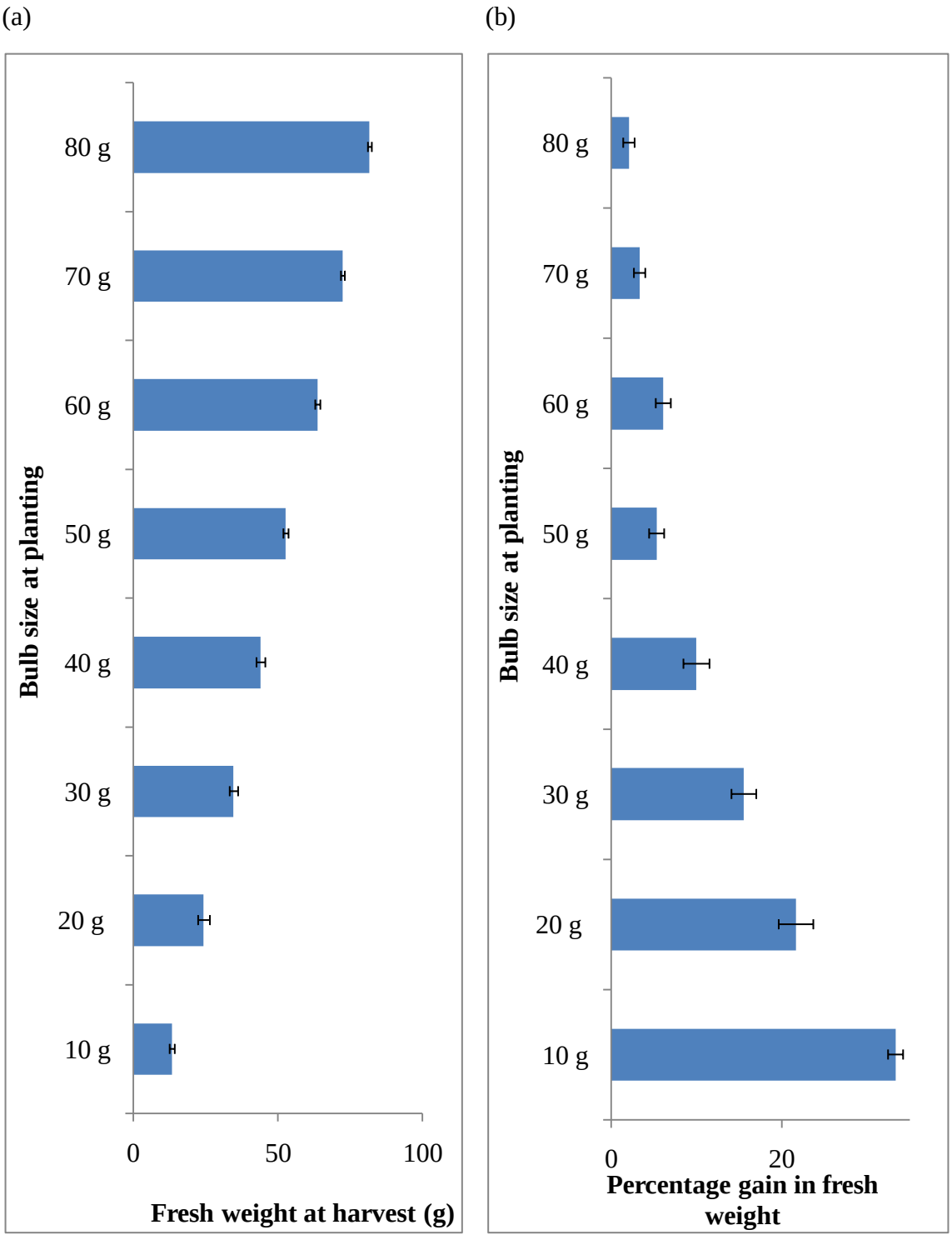


Fig 5.06: Influence of bulb size at planting of hyacinth on harvest yield, (a) Fresh weight of mother bulb at harvest and (b) percentage gain in fresh weight of the mother bulb at harvest. Bars represent mean values ± standard error of five replicates.

Table 5.1: Relationship among parameters measured on hyacinth in relation to bulb size at planting.

BS	1.00												
LW	0.75	1.00											
LL	0.47	0.88	1.00										
LA	0.68	0.96	0.92	1.00									
CC	0.94	0.72	0.45	0.66	1.00								
IH	0.91	0.87	0.66	0.81	0.85	1.00							
ID	0.96	0.71	0.42	0.61	0.93	0.93	1.00						
IL	0.96	0.82	0.61	0.78	0.93	0.96	0.94	1.00					
NoF	0.95	0.58	0.25	0.52	0.94	0.81	0.93	0.89	1.00				
ISD	0.98	0.67	0.39	0.61	0.92	0.88	0.95	0.94	0.97	1.00			
NoB	0.39	0.82	0.88	0.79	0.31	0.58	0.37	0.49	0.21	0.35	1.00		
FB	0.81	0.94	0.76	0.86	0.72	0.86	0.78	0.81	0.64	0.72	0.77	1.00	
BHW	0.98	0.76	0.49	0.70	0.94	0.92	0.96	0.97	0.95	0.98	0.40	0.80	1.00
	BS	LW	LL	LA	CC	IH	ID	IL	NoF	ISD	NoB	FB	BHW

The abbreviations BS, LW, LL, LA, CC, IH, ID, IL, NoF, ISD, NoB, FB, BHW refer to bulb size, leaf width, leaf length, leaf area, chlorophyll content, inflorescence height, inflorescence diameter, inflorescence length, number of florets, inflorescence stalk diameter, number of bulblets (daughter bulbs), fresh weight of bulblets and mother bulb harvest weight respectively. Values are correlation coefficients (r) between any two of the parameters measured.

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5.2.1.2 The lily

Studies on effects of bulb size at planting on growth and yield of the lily bulb were conducted between April and November 2009. In this study, the lily bulbs were categorised into four bulb sized groups in terms of their fresh weight at harvest: 10-19, 20-29, 30-39 and 40-49 g. Results from this study did not differ significantly from those of the hyacinth experiment because the vegetative growth as well as all other parameters measured increased with increased bulb size at planting. This is also confirmed by the high correlation coefficient values (Table 5.2) between bulb size at planting and the measured parameters. Rate of leaf growth and plant height were high from 4 to 6 weeks after planting but between 6 and 12 weeks after planting growth rate was low. From 12 to 18 weeks after planting, growth had almost stopped as the increase in growth was not significant (Fig 5.07 a, b, c and d). For plants grown from the small bulbs (10-19 g bulb sizes), values of leaf width, leaf length, leaf area and plant height, at 4 weeks after planting were 7.56, cm, 22.89 cm, 20.56 cm^2 and 0.83 cm, respectively, whilst at 18 weeks after planting, values of these parameters were 83.93 cm, 556.80 cm, 617.81 cm^2 and 41.69 cm. These represent relative growth rates of 0.779 cm day^{-1}, 5.448 cm day^{-1}, 6.094 cm^2 day^{-1} and 0.417 cm day^{-1}, respectively. For plants obtained from the large bulbs (40-49 g bulb sizes), total leaf width, length, area and plant height were 55.32 cm, 250.7 cm, 248.02 cm^2 and 10.50 cm at 4 weeks after planting, whilst at 18 weeks after planting, the values were 218.15 cm, 1374.5 cm, 1589.50 cm^2 and 62.20 cm, respectively, giving relative growth rates of 1.662 cm day^{-1}, 11.467 cm day^{-1}, 13.689 cm^2 day^{-1} and 0.528 cm day^{-1} respectively. Flower quality (number of florets, florets length and peduncle length) also increased with increasing bulb size at planting and in fact, the difference between values of plants produced from the small and large bulbs of these parameters was vast. For instance, plants grown from the small bulbs (10-19 g) recorded average values of 1.17 cm, 6.08 cm and 1.95 cm for number of florets, floret length and peduncle lengths respectively, while those from the large bulbs (40-49 g) had 10.83, 17 cm and 9.83 cm of these parameters respectively (Fig 5.08 a, b and c). However, at 14 weeks after planting, the plants from the large bulbs aborted a higher percentage of florets than did those from the small bulbs (Fig 5.08 d). As was observed in the case of hyacinth, chlorophyll concentration of plants from the large bulbs of the lily was higher than those of the small bulb, but in general, the reduction in chlorophyll content from 12 weeks after planting to 18 weeks after planting was higher for plants obtained from large bulbs than the small bulbs. A similar observation was made with

the rate of photosynthesis and stomatal content (Fig 5.09 a, b and c). Fresh weight of the mother bulb at harvest as well as the percentage gain in weight followed the same distribution pattern as that of hyacinth (Fig 5.10 a). But unlike the case of hyacinth, both the fresh weight and number of daughter bulbs (bulblets) observed at harvest increased with increasing bulb size at planting (Fig 5.10 b).

Table 5.2: Relationship between bulb size at planting and parameters measured of the lily. Values are correlation coefficients (r) between bulb size and parameters measured.

Parameter	Bulb size at planting
Leaf width	0.94
Leaf length	0.94
Leaf area	0.94
Plant height	0.88
Chlorophyll content	0.92
Floret length	0.94
Peduncle length	0.94
Number of florets	0.86
Stomatal conductance	0.94
Photosynthetic rate	0.94
Percentage of florets aborted	0.89
Number of offsets	0.91
Fresh weight of offsets	0.95
Bulb harvest weight	0.97

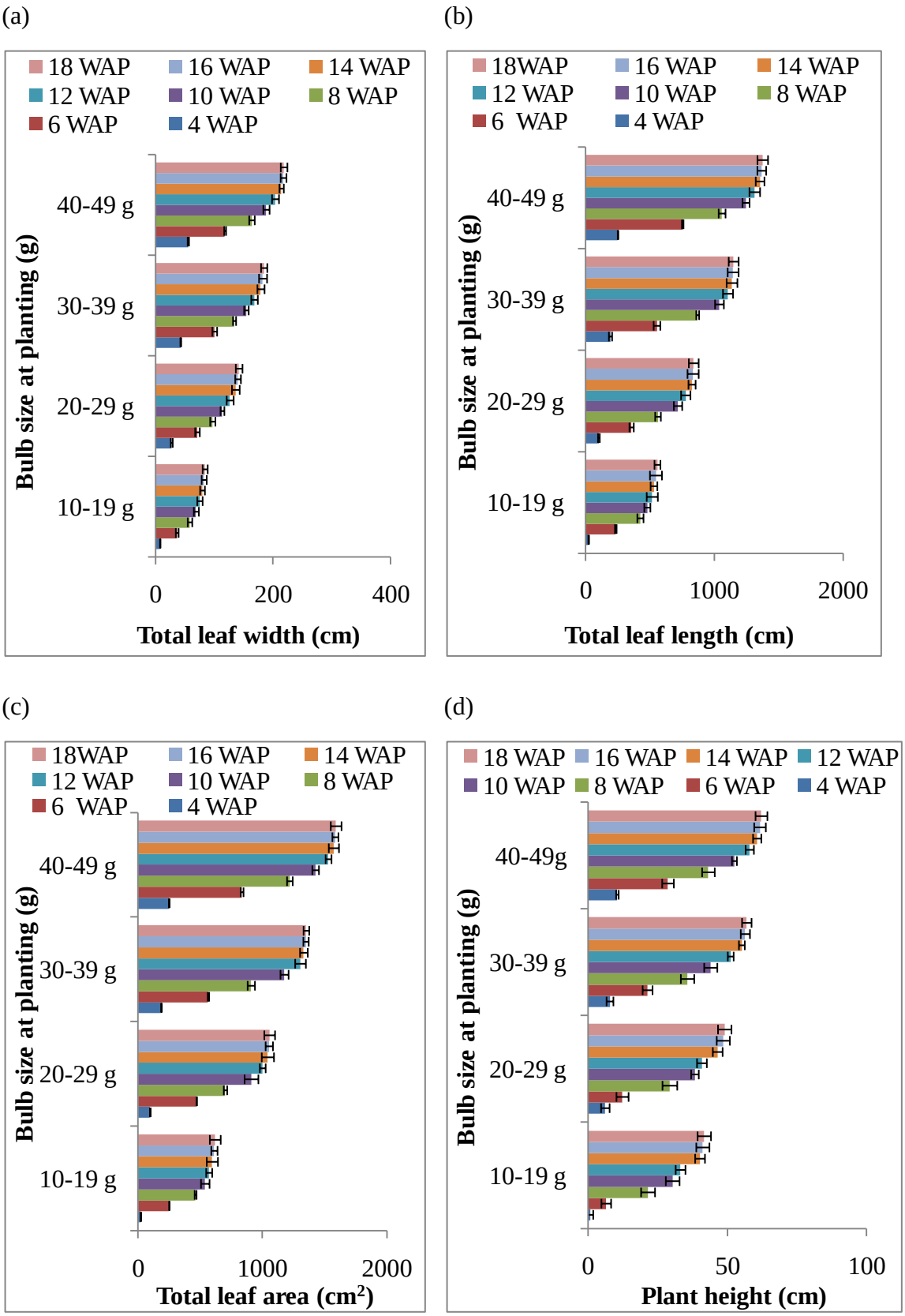


Fig 5.07: Impact of bulb size at planting on vegetative growth of the lily bulb, (a) total leaf width, (b) total leaf length, (c) total leaf area and (d) plant height. Values are means ± standard error calculated from three plants.

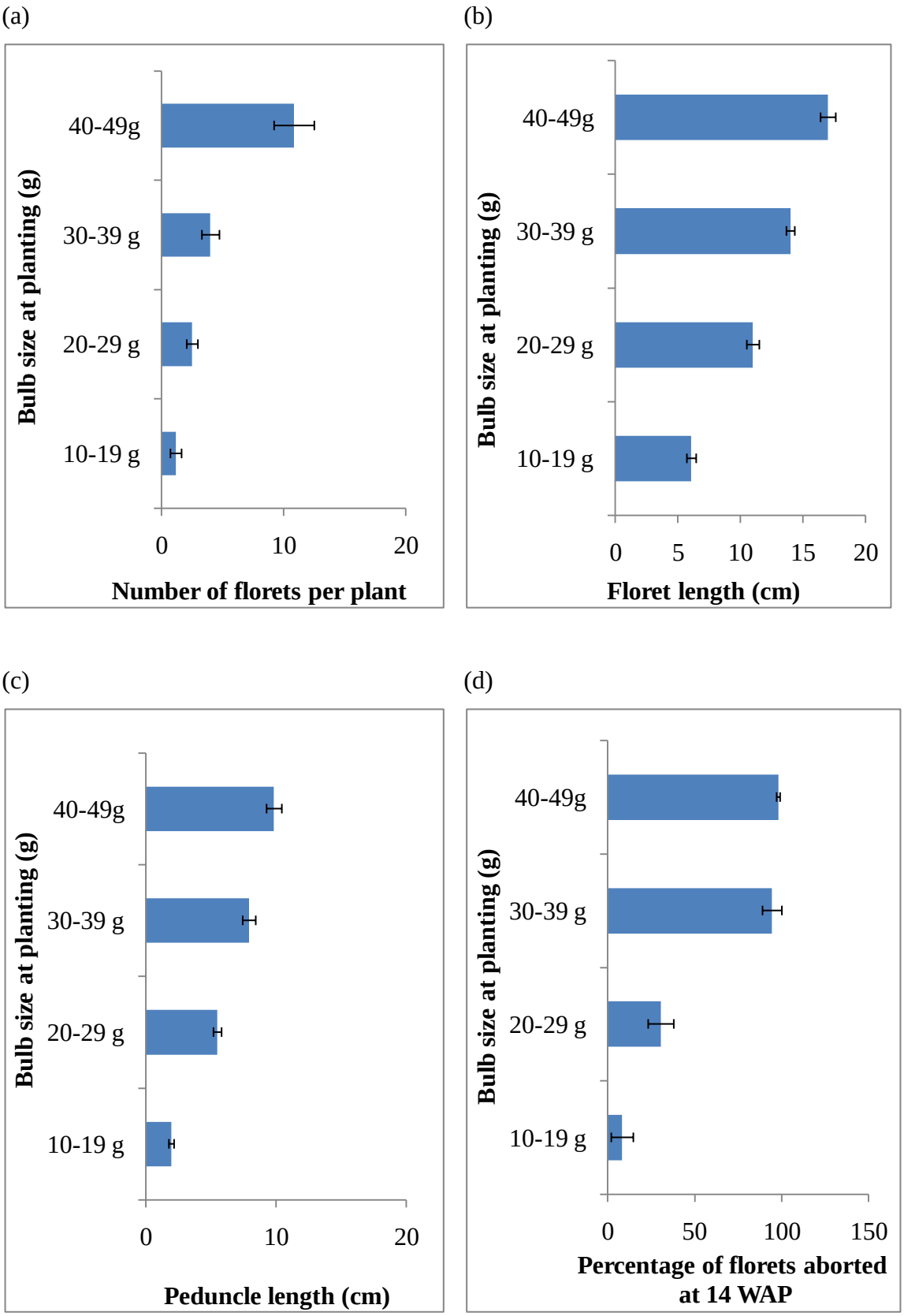
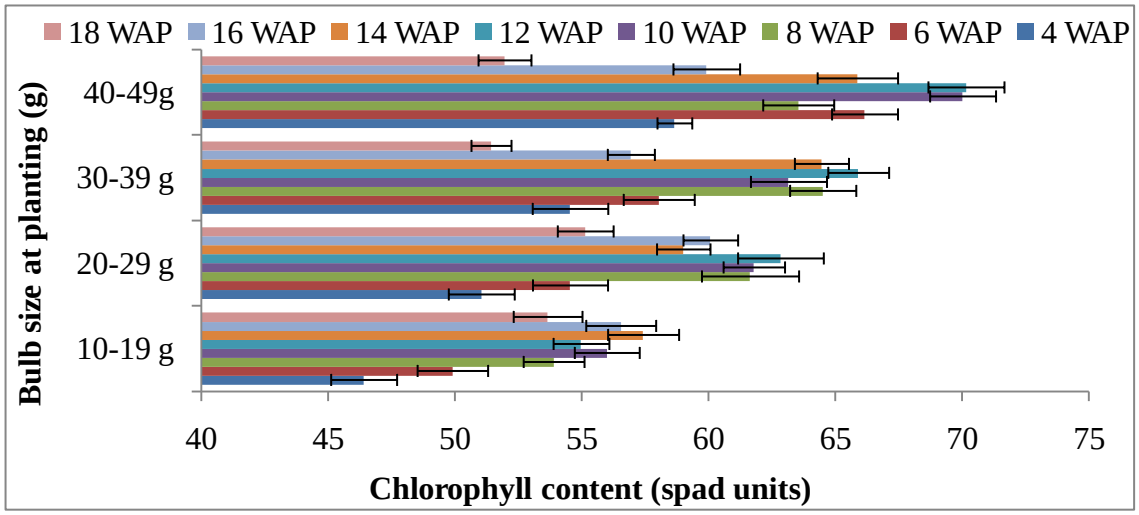
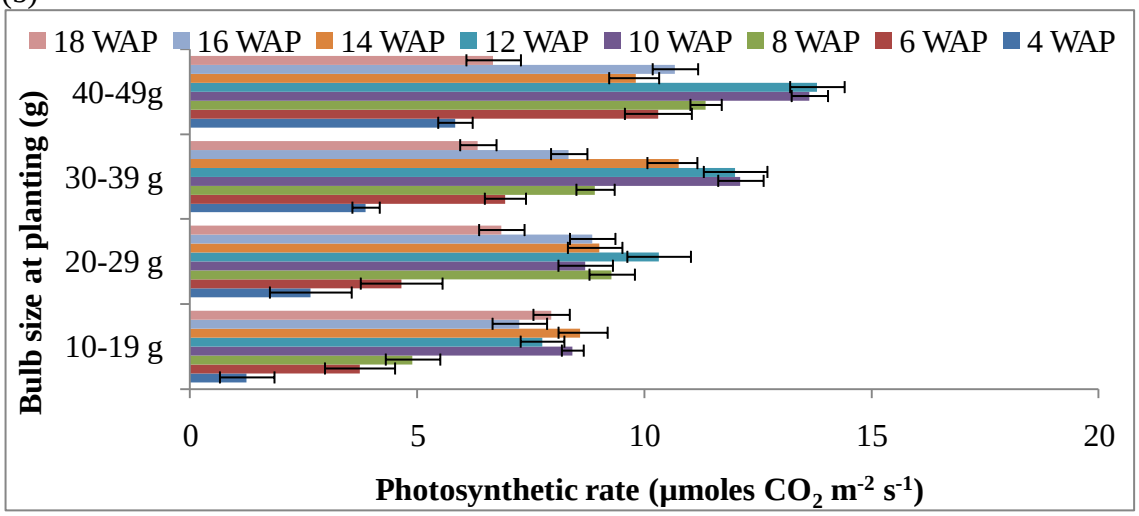


Fig 5.08: Effects of bulb size at planting on flower development of the lily bulb, (a) number of florets per plant, (b) floret length, (c) length of the peduncle, and (d) percentage abortion of florets. Results are mean values ± standard errors of three plants.

(a)



(b)



(c)

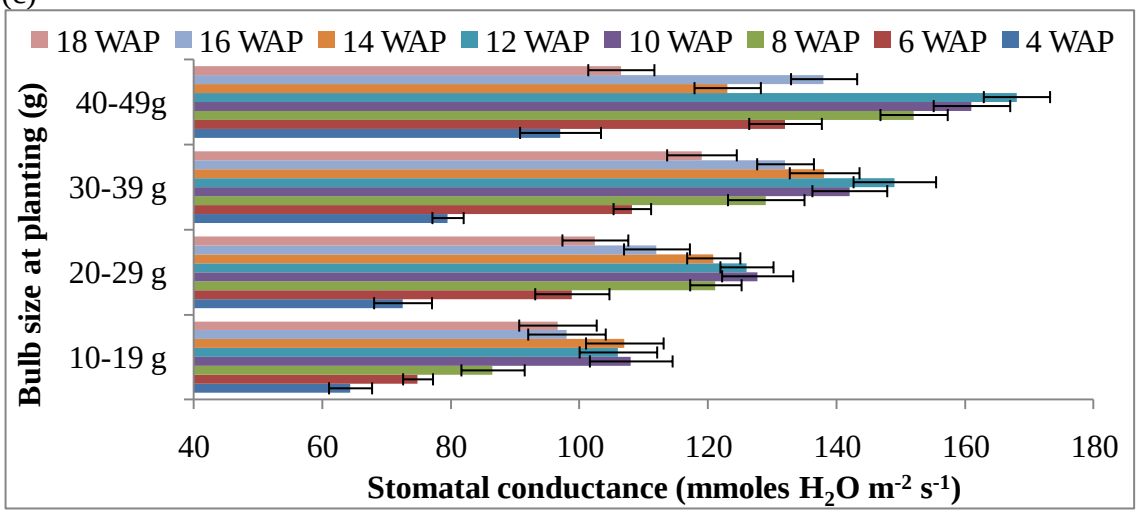
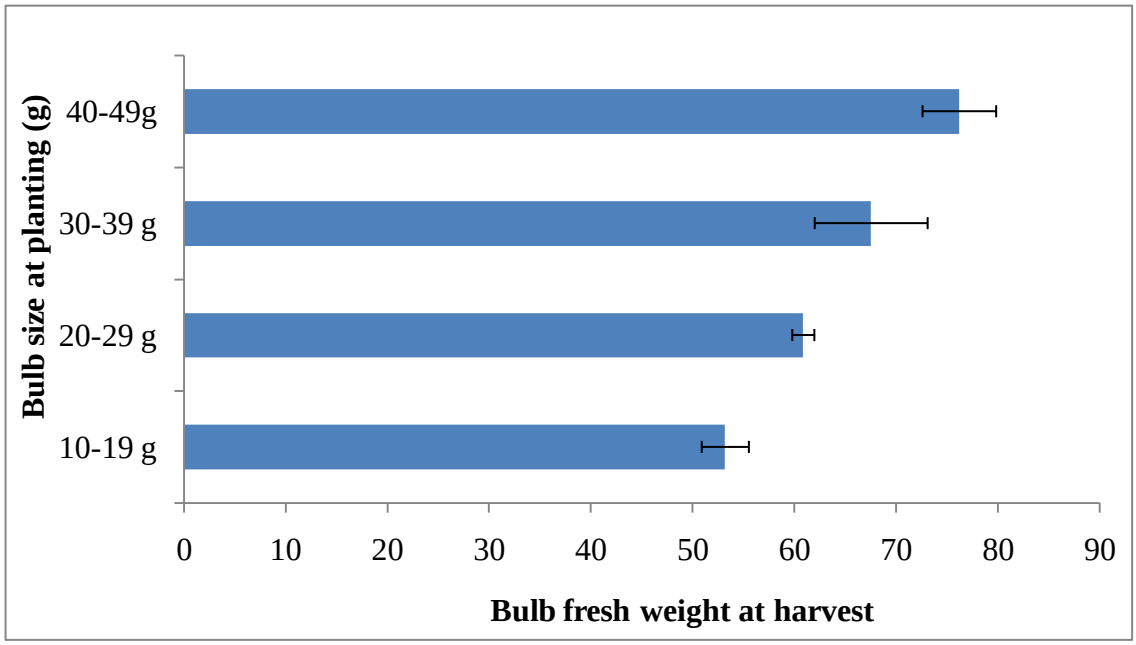


Fig 5.09: Bulb size at planting effects on (a) chlorophyll content, (b) photosynthetic rate and (c) stomatal conductance of the lily bulb. Values are means ± standard error of three plants.

(a)



(b)

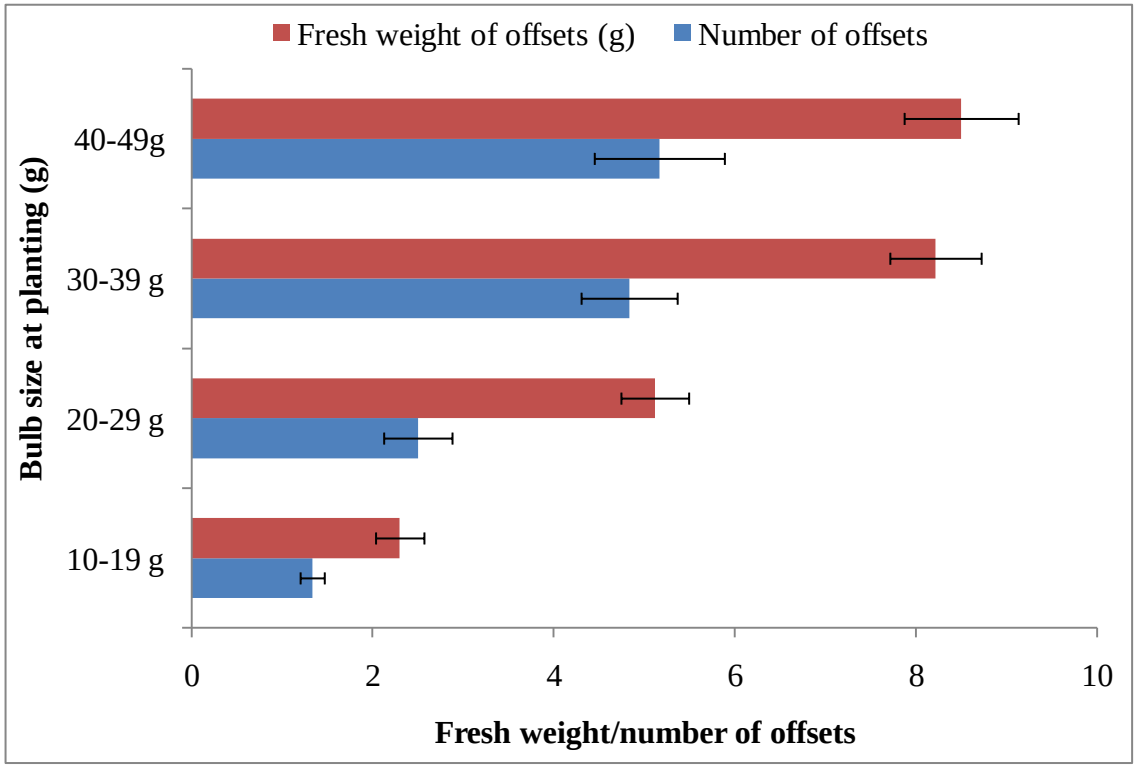


Fig 5.10: Influence of bulb size at planting on (a) fresh weight of the mother bulb and (b) offsets formation in the lily bulb. Results are the mean values ± standard errors calculated from three replicates.

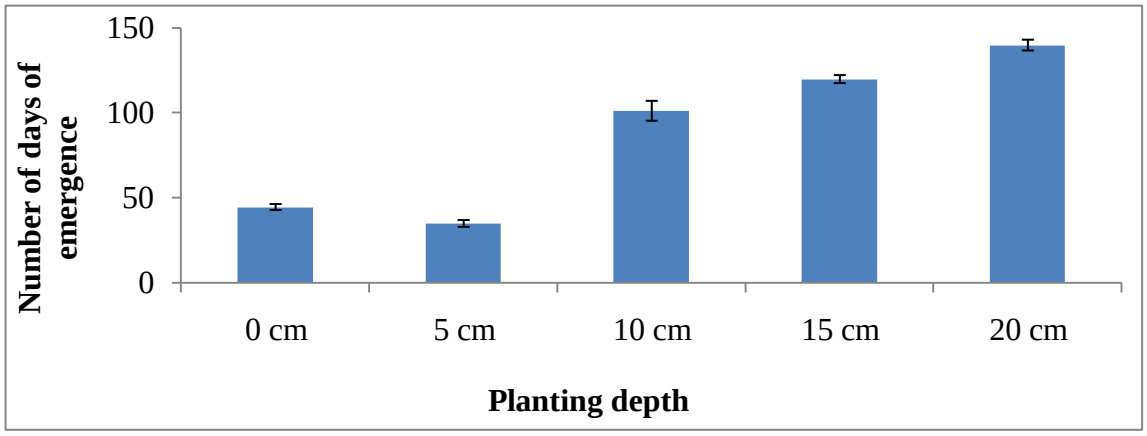
5.2.2 Response of the bulbs to planting depth

5.2.2.1 Hyacinth

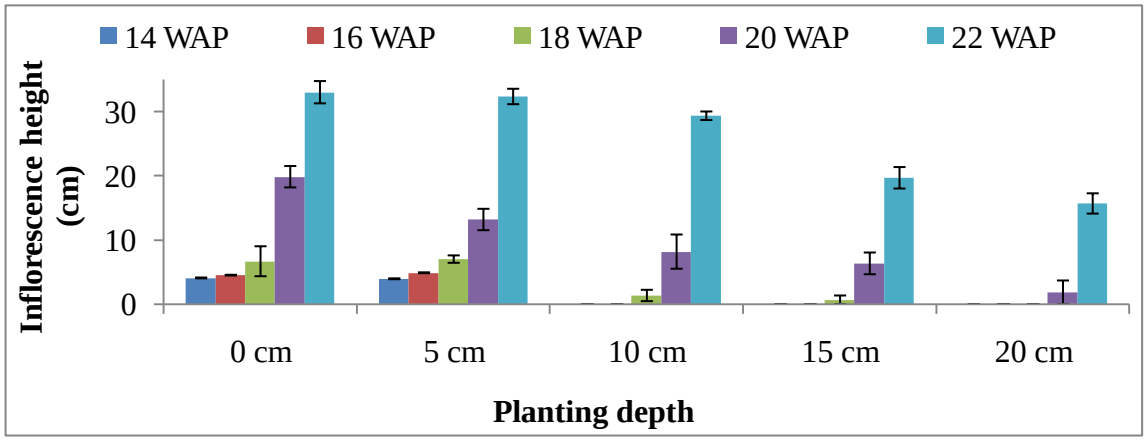
Studies on the influence of planting depth on growth and development of hyacinth bulb were carried out between October 2008 and June 2009. The bulbs were planted using the following five planting depths: 0, 5, 10, 15 and 20 cm from soil surface. The results from this experiment showed that significant differences existed among bulbs from the various planting depths with respect to their emergence (Fig 5.11a). In general, the deeper the depth of planting, the longer it took the bulbs to emerge from the soil after planting. Bulbs from the 5 cm depth regime emerged earlier, on average at 35 days after planting, followed by those from the 0 cm depth, on average at 44 days after planting, whilst bulbs from the 20 cm depth took the longest number of days (140 days) to emerge from the soil after planting. Planting depth had a tremendous impact also on inflorescence formation (Fig 5.11b) because the length and height of inflorescence decreased as the depth of planting increased. Values of these two parameters were similar for bulbs planted at 0 and 5 cm depth. In case of the bulbs planted at 10 and 15 cm depths, inflorescences were observed only after 18 weeks of growth; whilst in the case of bulbs belonging to the 20 cm depth regime, inflorescences were visible at 20 weeks after planting. The adverse effect of depth of planting on inflorescence formation of hyacinth is further illustrated by the photographs of some of the plants belonging to only part of these planting depth regimes (Fig 5.14a). Similarly, leaf growth characteristics (leaf width, length and area) were negatively affected by planting depth. Plants from the 5 cm depths displayed the highest value of leaf growth (Fig 5.12a, b and c) whilst those from the 20 cm depth gave the lowest. Generally, visible leaves were detected only after 18 weeks of growth (Fig 5.12b and c) in the case of plants from the 15 and 20 cm depth (deep planting regimes), and this dramatically reduced the growth of leaves in these regimes. Thus at 28 weeks after planting, leaf width were 31.07 cm, 34.61 cm, 32.90 cm, 21.97 cm and 16.01 cm respectively for the 0, 5, 10, 15 and 20 cm depths. In the same manner, the respective leaf length values at 28 weeks after planting were 196.67 cm, 264.59 cm, 253.42 cm, 155.67 cm and 123.83 cm whilst the values of leaf area at that time were 623.62 cm^2, 817.02 cm^2, 778.22 cm^2, 438.30 cm^2 and 331.99 cm^2, respectively, for the 0 cm, 5 cm, 10 cm, 15 cm and 20 cm depth. The development of chlorophyll followed a similar trend as leaf growth between 14 and 20 weeks after planting but later on, during the growth of the bulbs, plants from the 20 cm depth

recovered in chlorophyll content and at 28 weeks after planting their chlorophyll value slightly exceeded those from the other regimes. At harvest, the bulbs were found at depths of 2.83, 7.33, 12.1, 16.9 and 20.86 cm, respectively, for the 0, 5, 10, 15 and 20 cm planting depths, and these represent a downward movement of 2.83, 2.33, 2.10, 1.90 and 0.86 cm respectively (Fig 5.13b, Fig 5.15). This implies that the shallower the depth of planting, the greater the downward movement. Also at harvest, gain in fresh weight of the mother bulb (Fig 5.13c) was highest for bulbs planted at 5 cm depth, whilst those planted at 20 cm depth gave the least gain in weight. Bulbs planted at the 0 cm depth, however, did not give any gain in weight at harvest. The shoots of some of the bulbs planted at 0 and 20 cm depths still remained green (Fig 5.14b) even at 30-32 weeks after planting, that is, prior to harvesting, whereas those of the other planting regimes had died back indicating that the rate of senescence of the shoots delayed in the very shallow (0 cm) depth and the very deep (20 cm) depth.

(a)



(b)



(c)

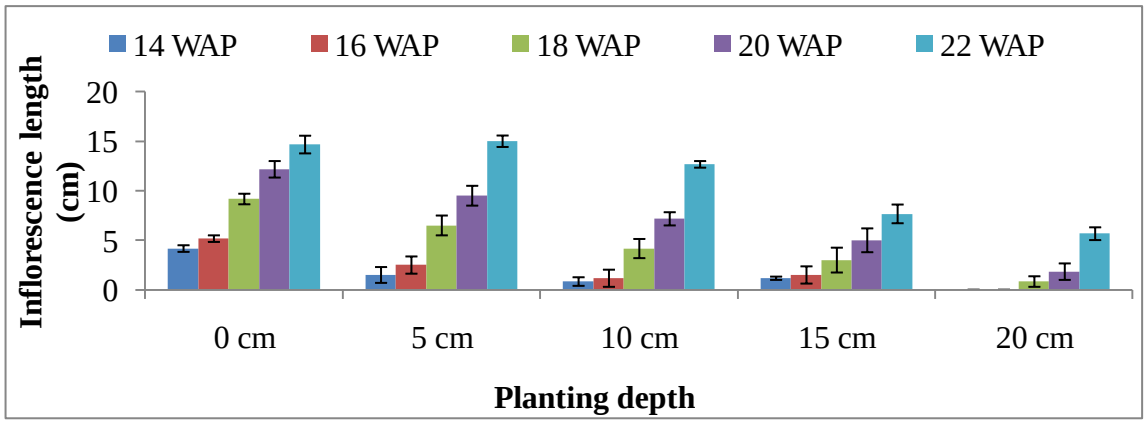
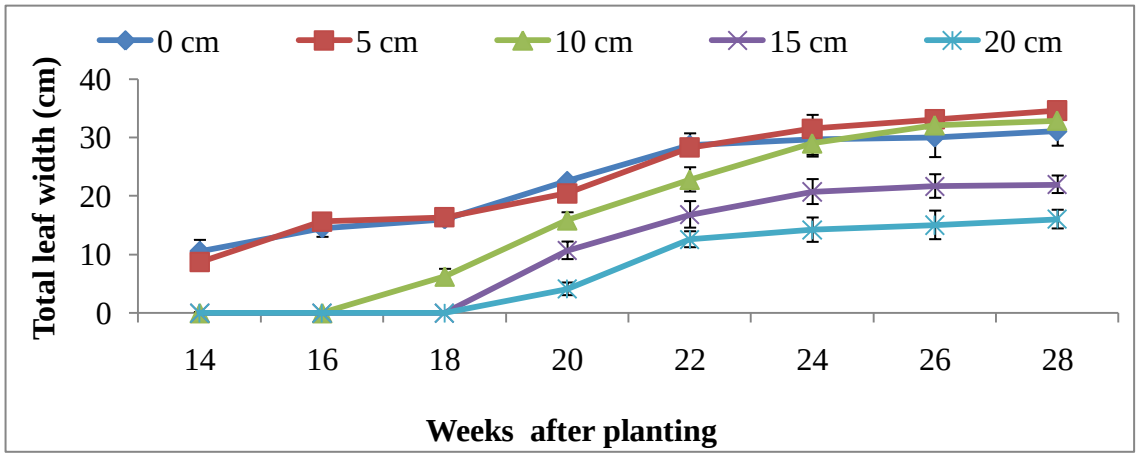
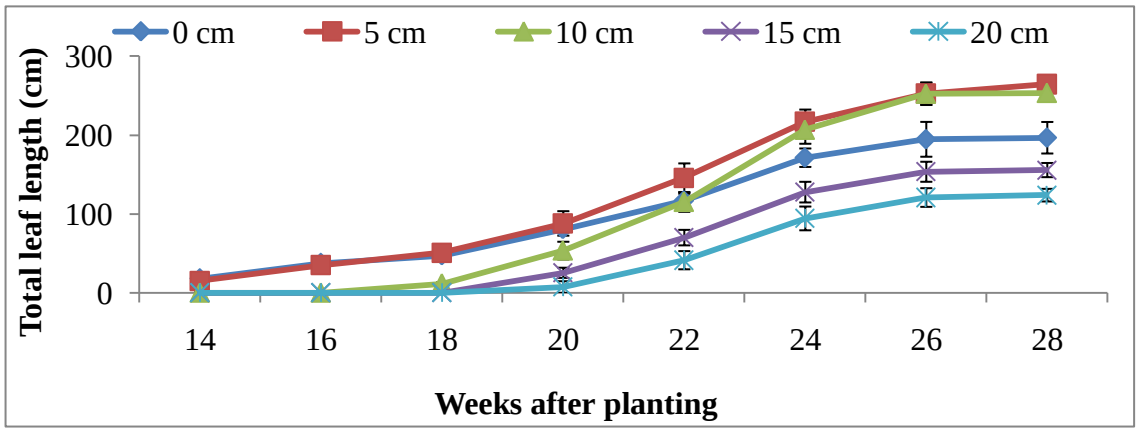


Fig 5.11: Influence of planting depth on emergence and inflorescence formation of hyacinth, (a) days of emergence, (b) inflorescence height and (c) inflorescence length. Results are mean values ± standard error from five plants.

(a)



(b)



(c)

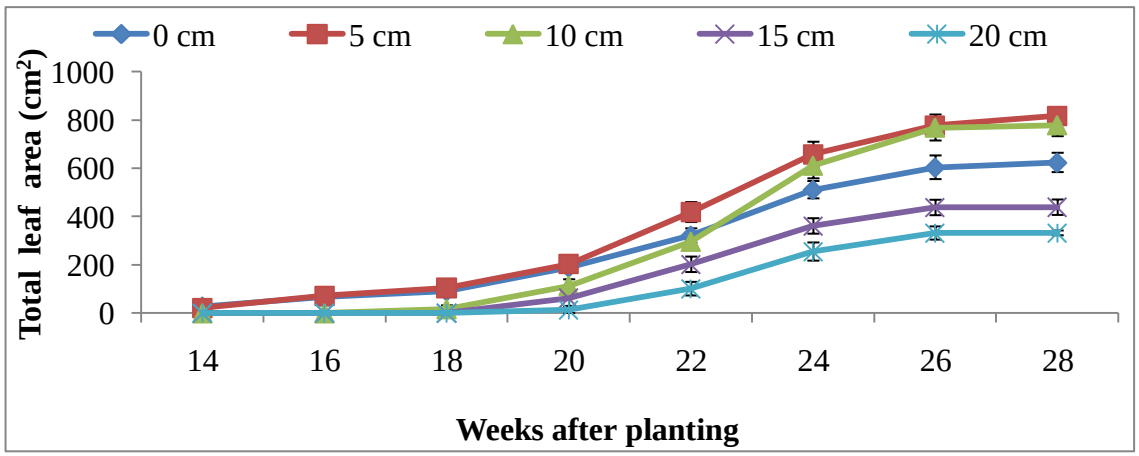
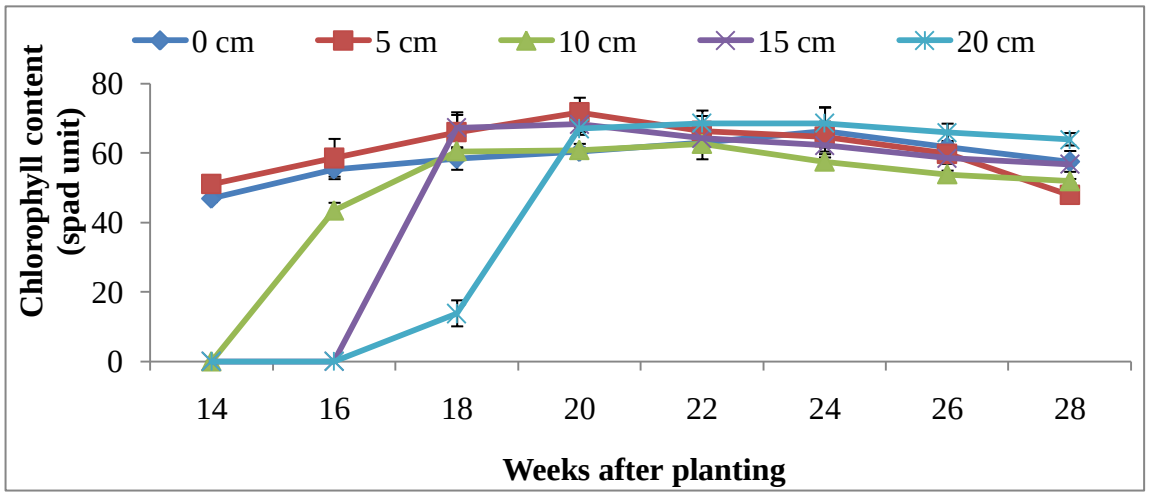
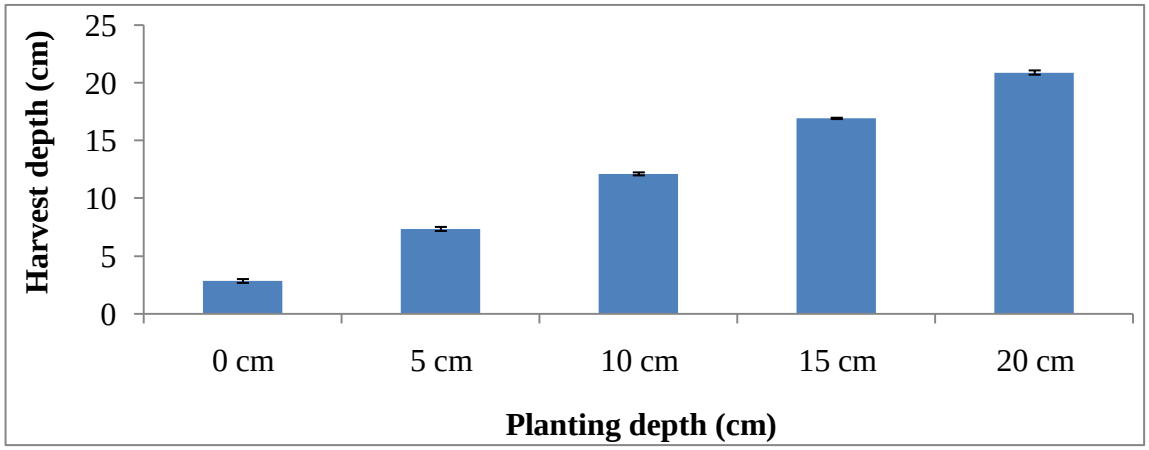


Fig 5.12: Impact of planting depth on leaf growth characteristics of hyacinth: (a) total leaf width, (b) total leaf length and (c) total leaf area. Values shown are means ± standard errors computed from five plants.

(a)



(b)



(c)

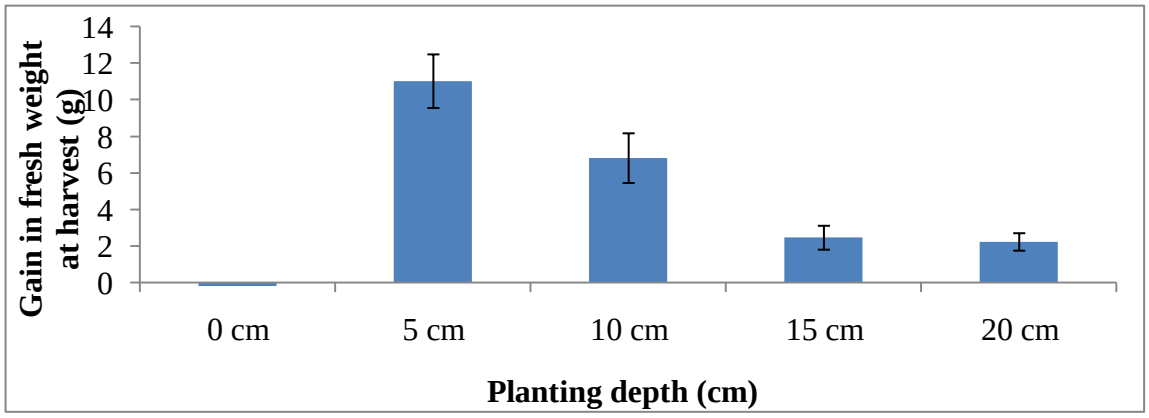


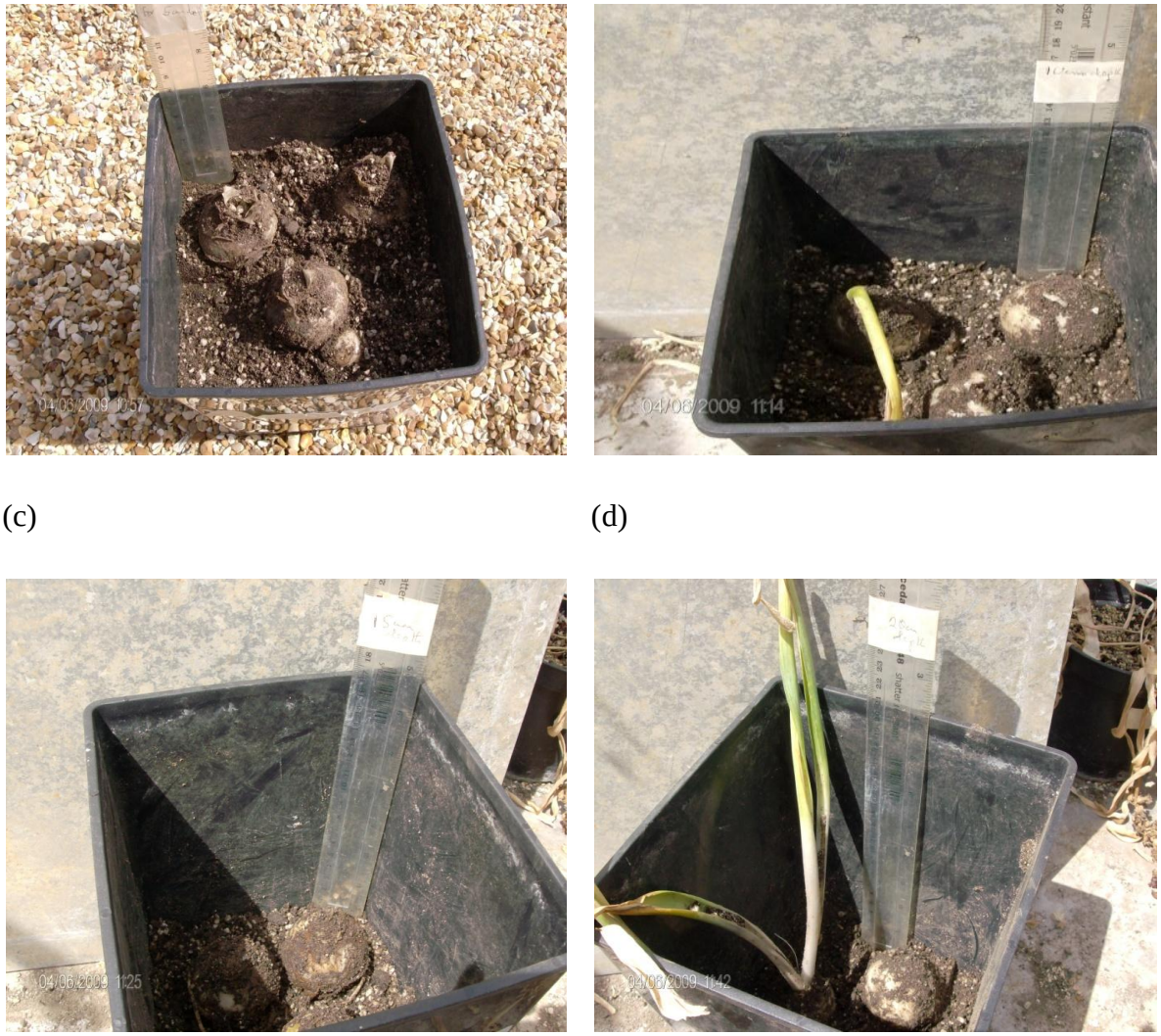
Fig 5.13: Effect of planting depth on (a) Chlorophyll content, (b) harvest depth and (c) gain in bulb yield of hyacinth. Bars represent the mean values ± standard error of five replicates. At harvest, plants from the 0 cm depth (c) did not yield any gain in weight.

(a)

(b)

Fig 5.14: Influence of planting depth on growth and senescence of hyacinth. Picture (a) shows plants at full flowering stage (22 WAP), those of the 0 cm depth on the extreme left, followed by those planted at 5 cm depth, whilst on the extreme right are those planted at the 20 cm depth. In (b), rate of senescence of hyacinth plants from bulbs planted at different depths are shown (starting from the extreme right, plants from 0, 5, 10, 15 and 20 cm depths respectively just before harvesting).

(a) (b)



(c) (d)

Fig 5.15: Photographs showing the depths at harvest of the hyacinth bulbs planted at 5 cm (a), 10 cm (b), 15 cm (c) and 20 cm (d). At harvest, the bulbs were at depths of 7.33, 12.10, 16.90 and 20.86 cm respectively for the 5, 10, 15 and 20 cm planting depths.

5.2.2.2 The lily

Between April and November 2009, experiment on the influence of planting depth on growth and development of the lily was also carried out at the same time as the effects of bulb size at planting on growth was conducted. As in hyacinth, the lily bulbs were planted at 0, 5, 10, 15 and 20 cm depths with a view to comparing their growth and development. Unlike the case of hyacinth experiment where even placing the bulbs on soil surface (0 cm) resulted not only in sprouting but also inflorescence formation and leaf growth, the lily bulbs that were planted at 0 cm depth (placed at soil surface) neither emerged nor survived. As was observed in the case of hyacinth, days of emergence after planting increased with increasing depth of planting (Fig 5.16a). Bulbs planted at 5 cm depth emerged first, on average at 12 days after planting, whilst those planted at 20 cm depth took on average 35 days to emerge from the soil after planting. In general, chlorophyll content decreased with increasing planting depth, but later in the season, bulbs planted at 15 and 20 cm (deep planting regimes) made a recovery in terms of chlorophyll development until there were no significant differences among the treatments at 18 weeks after planting (Fig 5.16b). Leaf growth (leaf width, leaf length and leaf area) and also growth in plant height were rapid between 4 and 8 weeks after planting, but the growth in these parameters reduced after this period, and growth assumed a plateau from 12 to 18 weeks after planting (Fig 5.16c, d, e and f). The trend in flower development and characteristics (length of florets and peduncle) was similar to that of hyacinth but the percentage of flower abortion was higher with plants from the shallow planting than those from deep planting (Fig 5.17a, b and c). As was observed in the case of hyacinth, the shallower the depth of planting, the greater the movement of the bulbs at harvest (Fig 5.18a, Fig 5.19). The gain in weight of the mother bulb at harvest (Fig 5.18b) similarly followed the same distribution pattern as the hyacinth experiment.

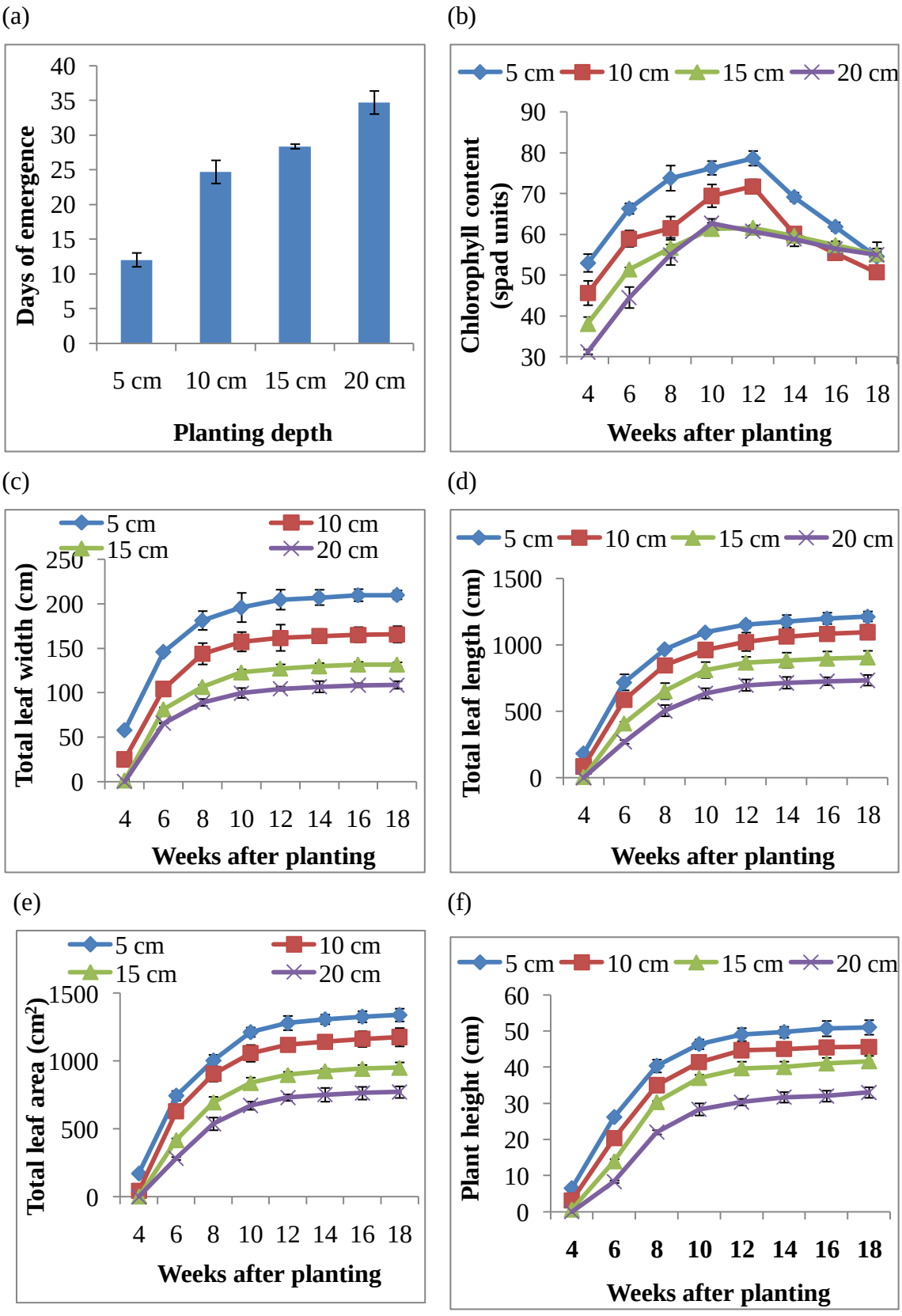
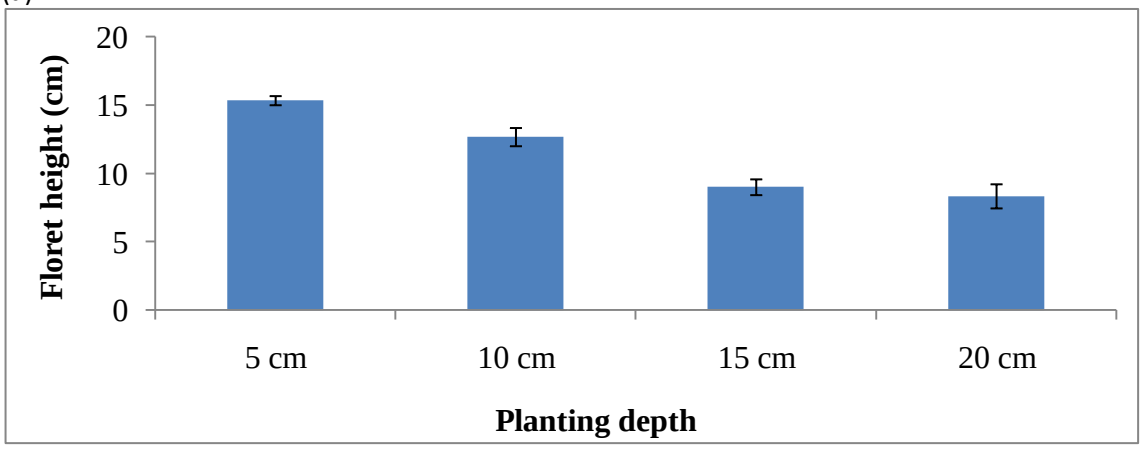
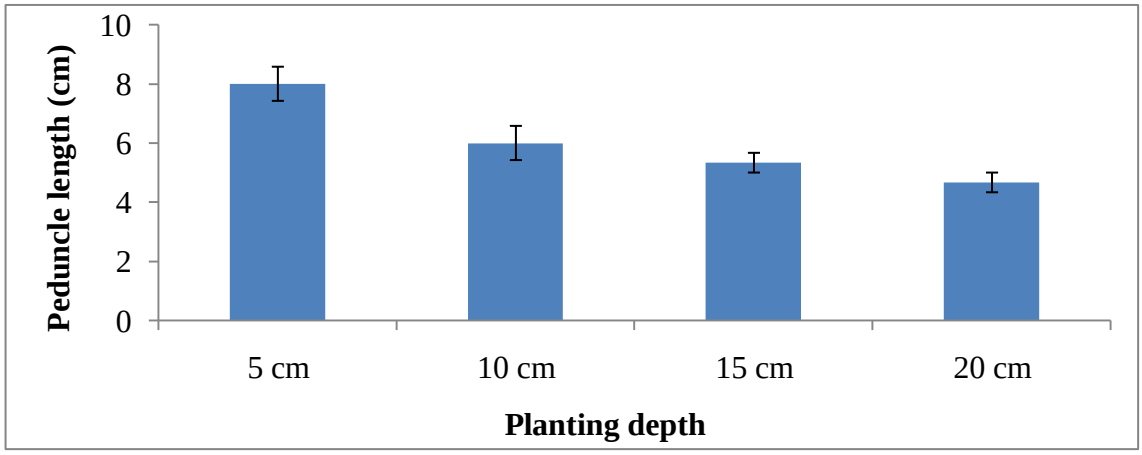


Fig 5.16: Influence of planting depth on sprouting, chlorophyll development and vegetative growth characteristics of the lily bulb; (a) emergence, (b) chlorophyll content, (c) total leaf width, (d) total leaf length, (e) total leaf area and (f) plant height of the lily. Bars represent mean values ± standard error calculated from three plants.

(a)



(b)



(c)

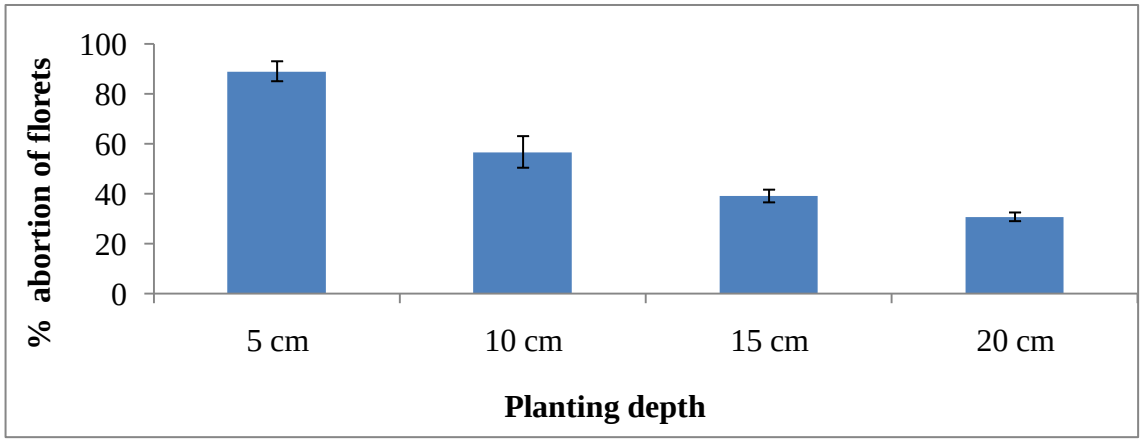
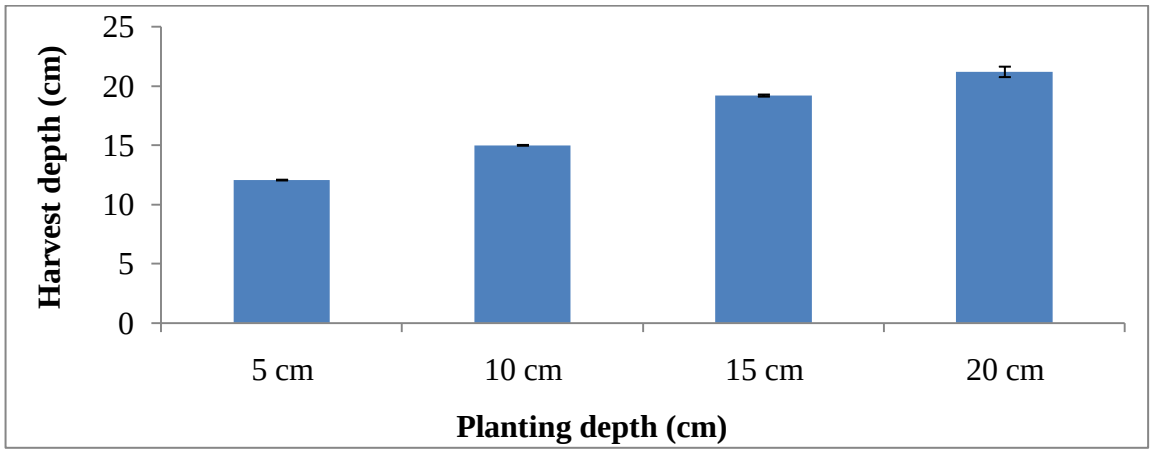


Fig 5.17: Effect of planting depth on flower development, harvest depth and yield of the lily bulb, (a) floret height, (b) peduncle length, (c) flower abortion. Bars represent mean values ± standard error computed from three replicates.

(a)



(b)

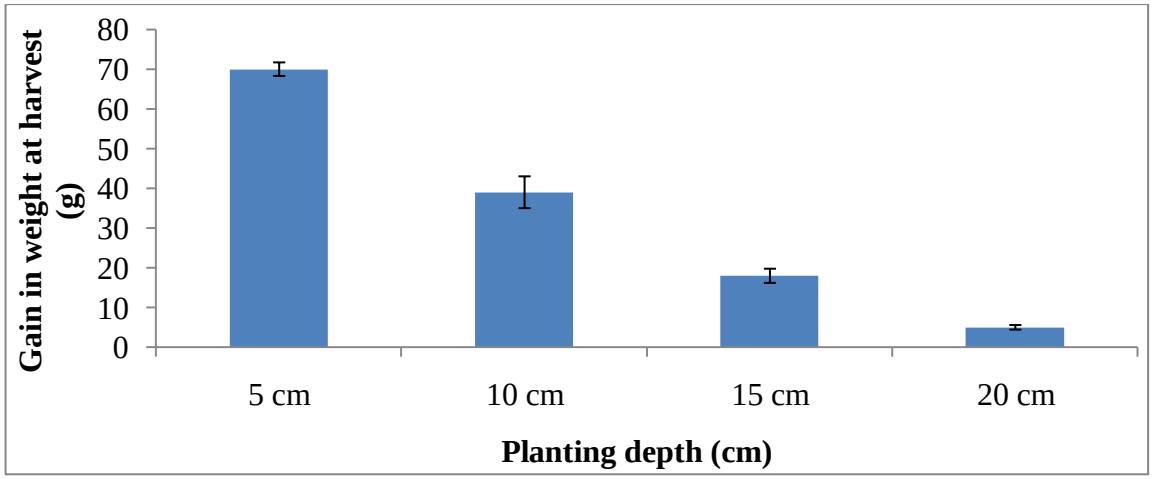


Fig 5.18: Effect of planting depth on (a) harvest depth and (b) gain in weight at harvest. Planting the lily bulb at 0 cm depth (i.e. placing the bulb at soil surface) led to the death of the bulb, hence no data for this treatment. Bars represent mean values ± standard error computed from three replicates.

(a) (b)

(c) (d)

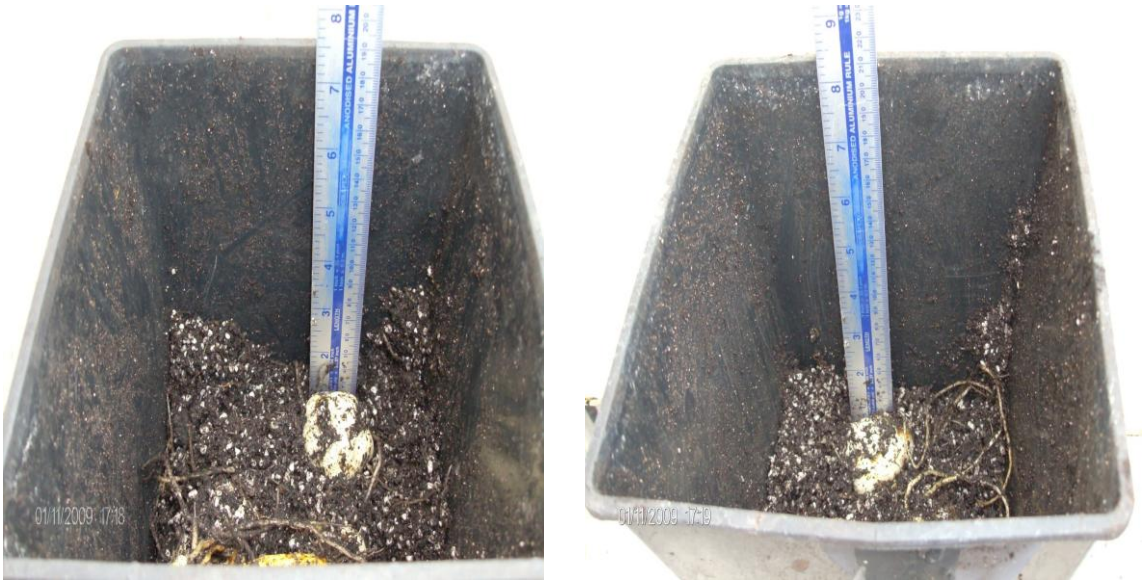


Fig 5.19: Photographs showing depths during harvesting of the lily bulb. Plants produced from bulbs that were planted at 5 cm (a) recorded an average depth of 12 cm at harvest; those planted at 10 cm (b) were at 15 cm at harvest, bulbs from the 15 cm planting depth (c) were harvested at depth of 19 cm whilst those from the 20 cm depth (d) were at 21 cm at harvest.

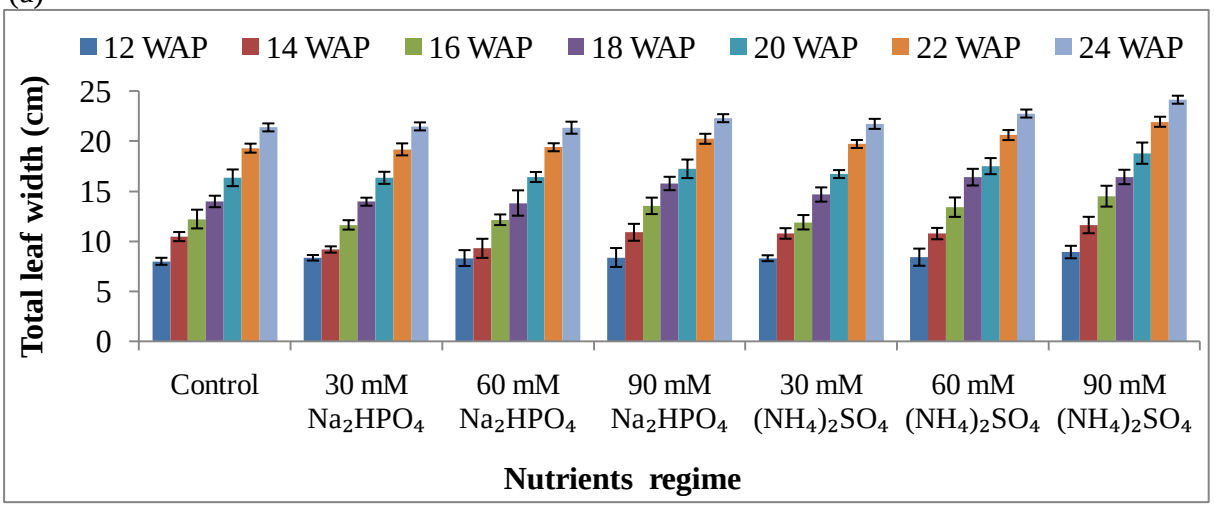
5.2.3 Bulbs and nutrients supply

5.2.3.1 Hyacinth

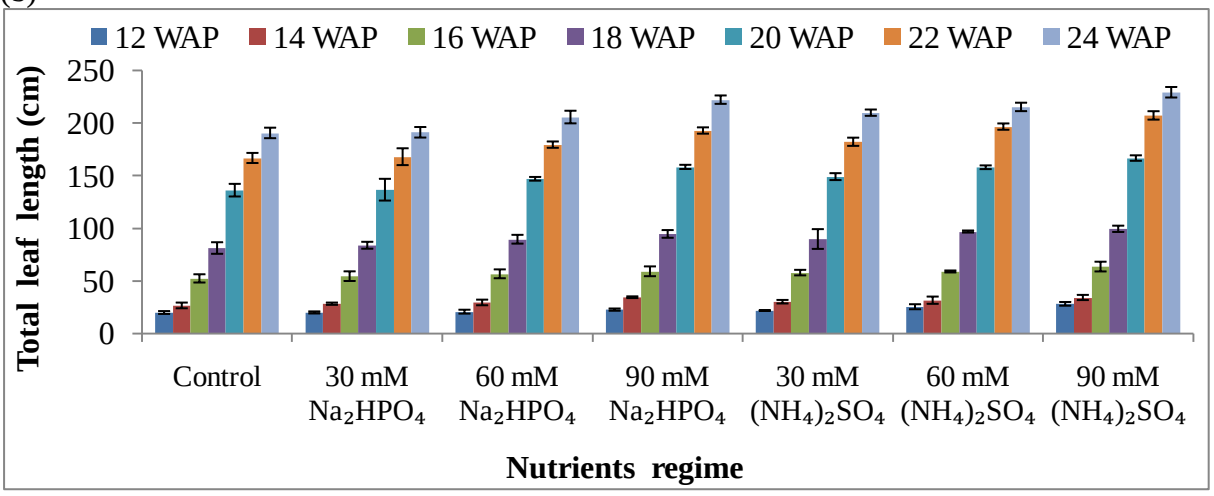
Results of Chapter 3 indicated that the reserved carbohydrates, basically starch of the bulb scales (particularly that of the outer scales) decreased to about 50 % of the initial value at about one month after planting of the hyacinth bulbs. This decrease mainly coincided with the time of sprouting of the bulbs. Since in geophytes and for that matter flower bulbs, the initial growth and development depends on the reserves stored in the bulbs, a reduction in the stored reserves, following sprouting and emergence, will have major consequences on the subsequent growth and development of the plant. Studies were therefore conducted with a view to replenishing the nutrients (starch) lost by the bulb prior to, and during emergence. Thus between October 2008 and June 2009, a study on the response of hyacinth bulb to nutrients supply was carried out. In this experiment, three levels (30, 60 and 90 mM) each of ammonium sulphate [$(NH_4)_2SO_4$], dibasic sodium hydrogen phosphate [Na_2HPO_4] and sodium sulphate [Na_2SO_4] were applied to the plants on weekly basis from 8 to 20 weeks after planting. Plants that did not receive any nutrients application were considered as control. Results from this study revealed that the supply of the N and P-based nutrients slightly increased leaf growth. Particularly, leaf length and area of hyacinth increased following the application of either 60 or 90 mM Na_2HPO_4 or $(NH_4)_2SO_4$ as compared with the control. However, plants responded more positively to the application of $(NH_4)_2SO_4$ than Na_2HPO_4 in terms of leaf growth because plants fed with the former recorded higher increases in leaf growth than those that received application from the latter (Fig 5.20). On the contrary, the application of Na_2HPO_4 at 60 or 90 mM enhanced inflorescence development (inflorescence diameter, inflorescence height and length) more than did $(NH_4)_2SO_4$ at the same rates of application. But in general, the two nutrients outperformed the control in terms of inflorescence formation (Fig 5.21a, b and c). Plants fed with $(NH_4)_2SO_4$, even at the 30 mM level, produced higher chlorophyll content than those that received nutrients from the Na_2HPO_4 (Fig 5.22a) and the control. In general, chlorophyll content of plants fed with $(NH_4)_2SO_4$ increased as the level of the nutrients application increased. Plants to which Na_2HPO_4 was applied produced chlorophyll concentration that did not vary significantly from those of the control. The trend observed with gain in fresh weight at harvest (Fig 5.22b) as a result of the nutrients application mimicked the distribution pattern exhibited by leaf growth and chlorophyll formation, that is plants

that received nutrients from $(NH_4)_2SO_4$ produced the highest gain in fresh weight of the mother bulb at harvest, and especially those fed with 90 mM $(NH_4)_2SO_4$ were very outstanding. There was no clear cut relationship between nutrients application and number of bulblets formed (Fig 5.22c), though the fresh weights of offsets formed seemed to be higher as the level of applied $(NH_4)_2SO_4$ increased. Generally, plants fed with Na_2SO_4 produced similar responses as those of the control (Table 5.3a, b).

(a)



(b)



(c)

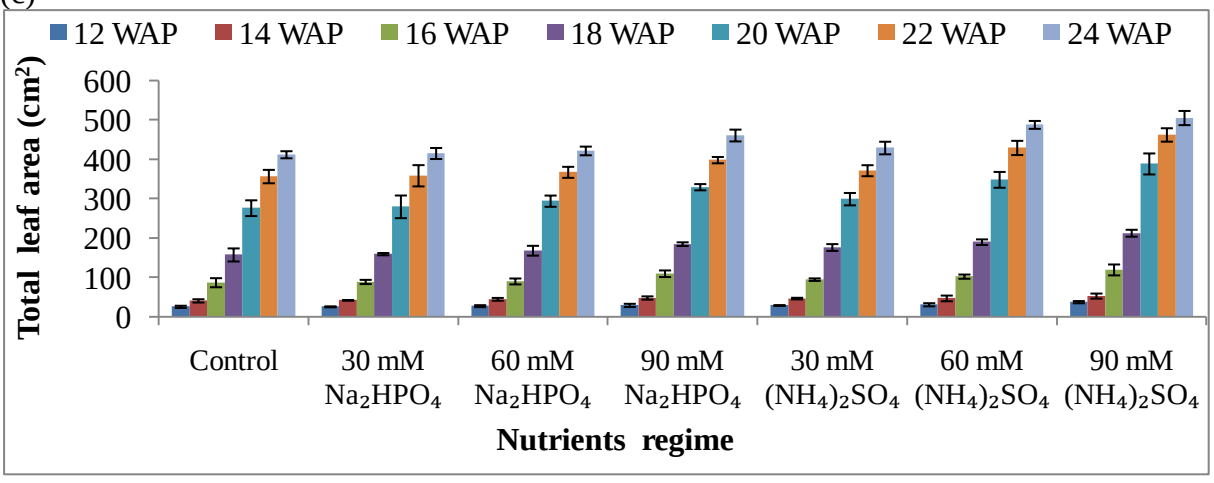
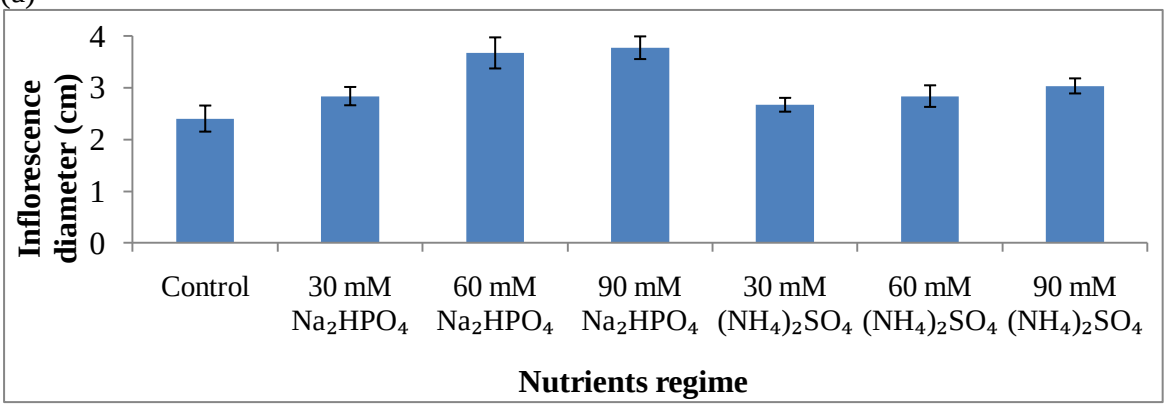
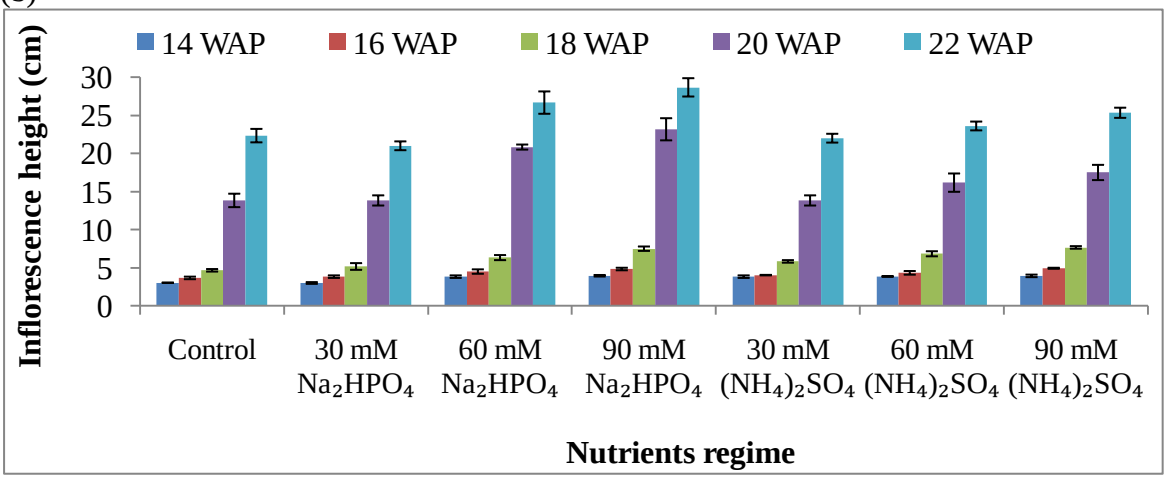


Fig 5.20: Influence of nutrients supply on leaf growth characteristics of hyacinth: (a) total leaf width, (b) total leaf length and (c) total leaf area. Results are mean values ± standard error of five plants.

(a)



(b)



(c)

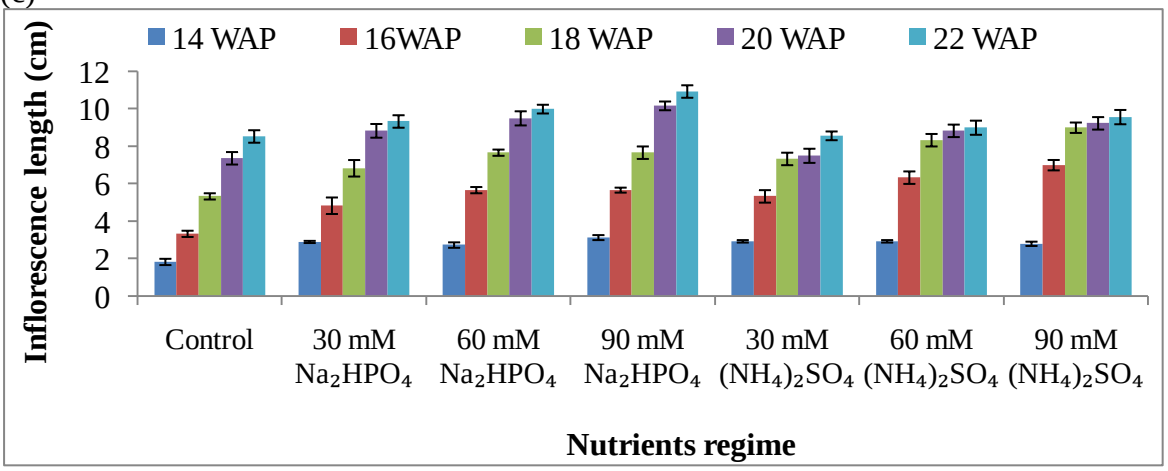
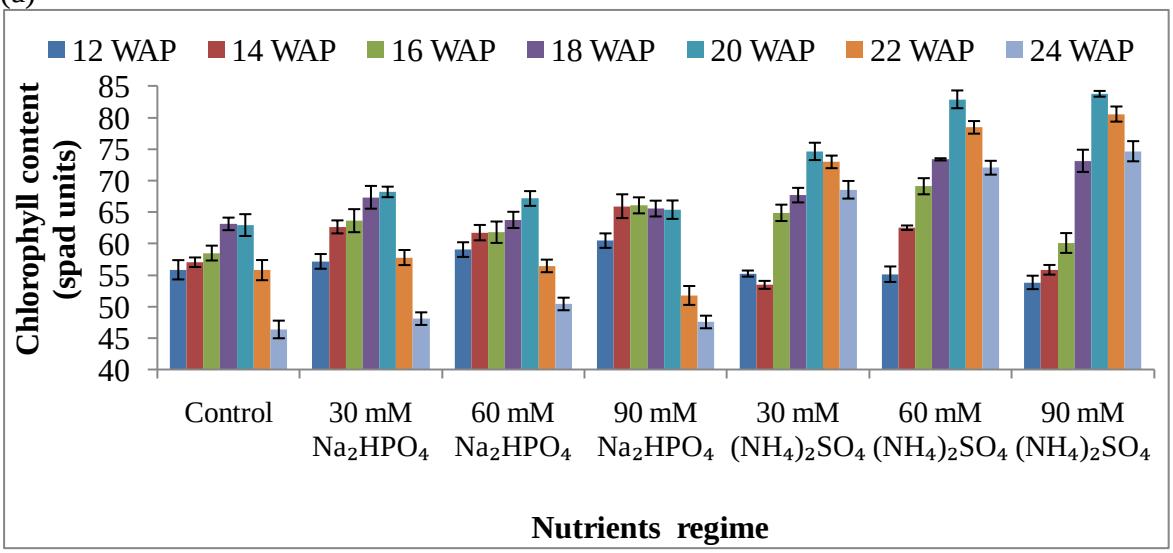
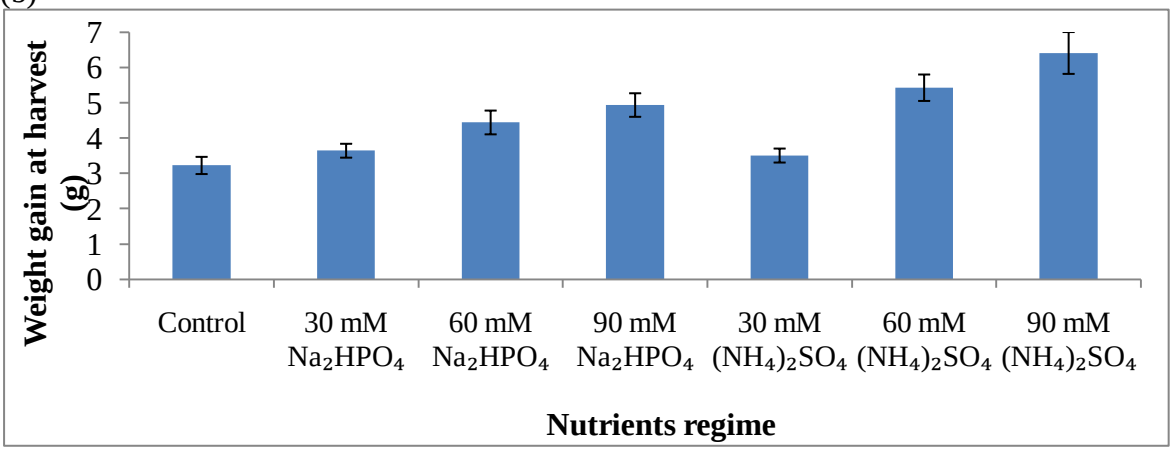


Fig 5.21: Effects of nutrients supply on inflorescence development of hyacinth, (a) inflorescence diameter, (b) inflorescence height and (c) inflorescence length. Results are mean values ± standard error computed from five plants.

(a)



(b)



(c)

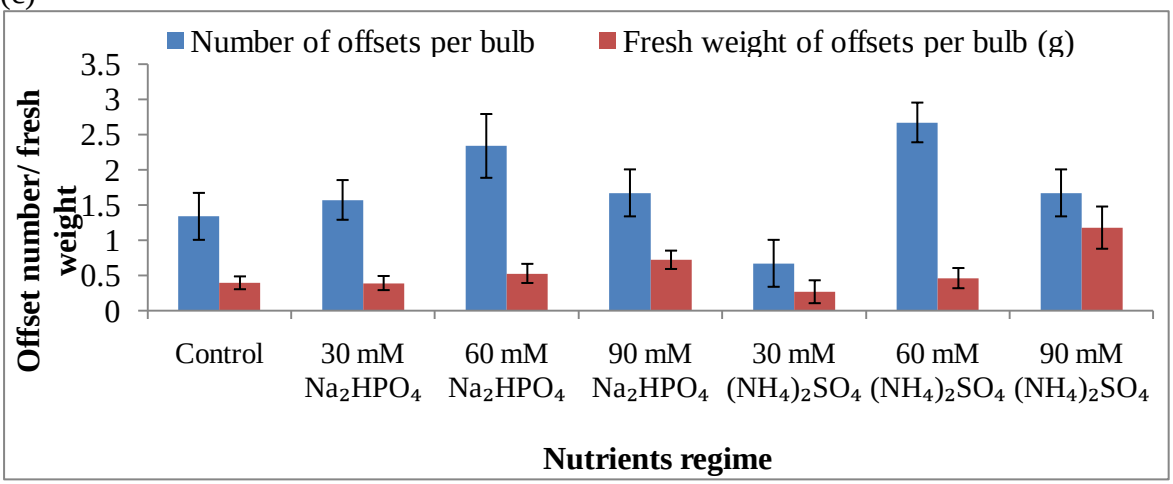


Fig 5.22: Influence of nutrients supply on: (a) chlorophyll content, (b) weight gain at harvest and (c) bulblets formation of the hyacinth. The Bars refer to mean values ± standard error of five replicates.

Table 5.3a: Leaf growth of hyacinth from the control and the Na_2SO_4 nutrient regime: (a) total leaf area, (b) total leaf length. Results are mean values ± standard error computed from five plants.

(a) total leaf area (cm^2)

	Weeks after planting						
Nutrients regime	12	14	16	18	20	22	24
Control	25.59 ± 2.84	40.74±4.14	86.91±11.51	157.44±16.59	276.15±19.98	356.45±17.01	411.81±9.00
30 mM Na_2SO_4	25.48 ± 1.05	40.44±1.79	83.99±6.83	155.81±6.87	275.88±34.79	353.62±32.10	402.03±21.01
60 mM Na_2SO_4	25.82 ± 1.37	42.89±1.76	86.55±7.91	164.84±5.45	277.23±17.32	353.49±16.03	408.12±15.02
90 mM Na_2SO_4	27.78 ± 1.15	43.72±3.49	89.91±7.67	165.66±12.55	281.60±10.45	359.64±10.02	412.04±8.02

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(b) total leaf length (cm)

Control	19.70±1.36	26.43±2.71	52.13±3.91	81.00±5.51	136.00±6.00	166.62±4.80	190.40±5.00
30 mM Na_2SO_4	19.63±0.61	25.63±0.93	51.83±2.52	80.66±1.20	133.66±3.48	162.75±12.90	181.93±8.00
60 mM Na_2SO_4	20.50±0.36	27.06±0.03	53.66±2.67	84.83±0.44	136.66±13.17	164.06±10.00	185.33±12.00
90 mM Na_2SO_4	21.90±0.35	29.03±0.87	54.43±2.72	86.66±11.20	138.00±3.61	172.24±13.00	194.80±9.00

Table 5.3b: Leaf growth and chlorophyll values of hyacinth from the control and the Na_2SO_4 nutrient regime: (c) total leaf width, (d) chlorophyll content. Results are mean values ± standard error computed from five plants.

(c) total leaf width (cm)

Control	8.00±1.05	10.46±0.75	12.22±1.49	13.97±1.57	16.33±0.83	18.29±0.45	22.36±0.40
30 mM Na_2SO_4	8.54±0.88	10.47±0.47	11.03±0.32	13.91±1.42	15.660.62	17.35±0.54	22.33±0.52
60 mM Na_2SO_4	8.41±0.80	9.51±0.55	11.01±1.39	14.57±0.75	16.71±0.81	18.33±0.40	22.91±0.50
90 mM Na_2SO_4	8.75±0.69	10.26±0.79	12.06±1.29	13.68±1.24	16.40±0.63	18.25±0.50	22.56±0.50

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(d) Chlorophyll content (spad units)

Control	55.83±1.53	57.83±1.36	58.48±1.18	63.12±1.30	62.53±1.73	54.78±1.61	46.36±1.40
30 mM Na_2SO_4	57.09±1.17	58.60±1.13	59.79±1.83	61.50±1.81	59.33±1.43	52.09±1.42	47.62±1.10
60 mM Na_2SO_4	57.30±1.16	59.27±1.22	58.93±1.70	63.14±1.29	60.28±1.17	51.86±1.36	45.78±1.12
90 mM Na_2SO_4	56.06±1.15	58.97±1.89	59.20±1.27	61.04±1.86	59.92±1.46	52.97±1.51	46.89±1.00

Table 5.3c: Inflorescence development, harvest data and bulblets formation in hyacinth from the control and the Na_2SO_4 nutrient regime: (a) inflorescence height, (b) inflorescence length, (c) inflorescence diameter and harvest data, and (d) number and fresh weight of offsets.

(a) inflorescence height (cm)

	Weeks after planting				
Nutrients regime	14	16	18	20	22
Control	3.03±0.03	3.66±0.17	4.66±0.16	13.83±0.88	22.33±0.88
30 mM Na_2SO_4	3.10±0.12	3.56±0.16	4.55±0.17	13.73±2.48	21.00±2.71
60 mM Na_2SO_4	3.16±0.29	3.50±0.03	4.63±0.18	13.65±1.33	23.66±0.89
90 mM Na_2SO_4	3.06±0.33	3.56±0.16	4.53±0.29	14.83±1.86	22.33±2.43

(b) inflorescence length (cm)

Control	1.83±0.16	3.33±0.17	5.33±0.16	7.36±0.53	8.53±0.33
30 mM Na_2SO_4	1.71± 0.21	3.26±0.16	5.41±0.17	8.06±0.73	9.12±0.35
60 mM Na_2SO_4	1.78±0.09	3.66±0.29	5.64±0.29	7.85±0.58	8.98±0.58
90 mM Na_2SO_4	1.83±0.19	3.36±0.29	5.45±0.28	8.13±0.33	9.26±0.88

(c) inflorescence diameter at 18 weeks after planting and gain in fresh weight at harvest

Nutrients regime	Inflorescence diameter (cm)	Gain in fresh weight (g)
Control	2.40 ± 0.25	3.22 ± 0.21
30 mM Na_2SO_4	2.41 ± 0.09	3.15 ± 0.17
60mM Na_2SO_4	2.38 ± 0.21	3.09 ± 0.15
90 mM Na_2SO_4	2.35 ± 0.19	3.12 ± 0.13

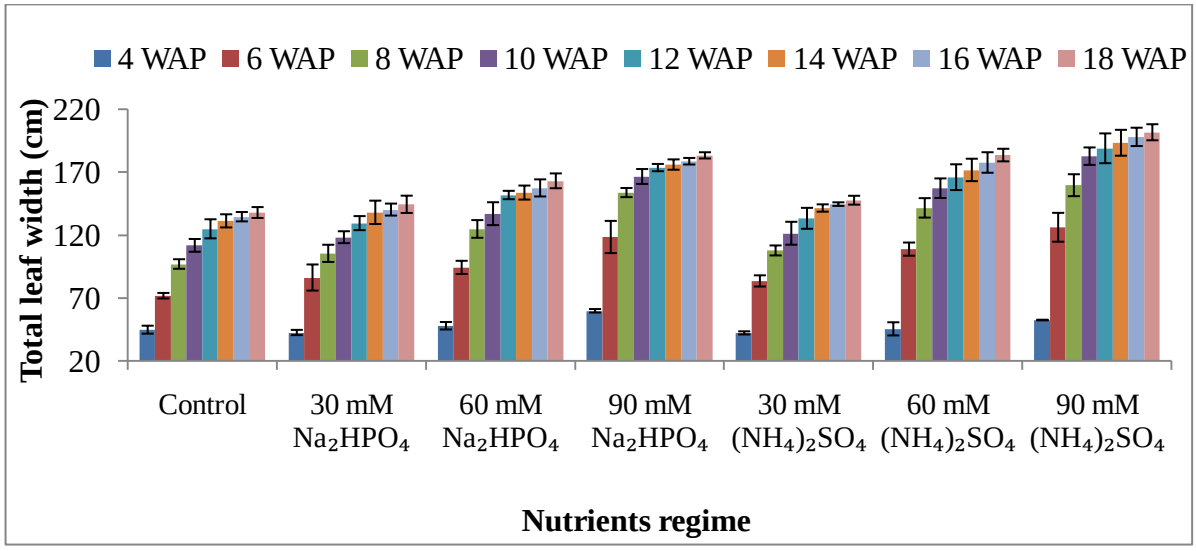
(d) Number and fresh weight of offset

Nutrients regime	Number of offsets per bulb	Fresh weight of offsets per bulb
Control	1.33 ± 0.33	0.39 ± 0.09
30 mM Na_2SO_4	0.96 ± 0.27	0.35 ± 0.03
60 mM Na_2SO_4	0.83 ± 0.19	0.29 ± 0.06
90 mM Na_2SO_4	1.13 ± 0.12	0.36 ± 0.11

5.2.3.2 The lily

The influence of nutrients supply on growth and productivity of the lily bulb was investigated at the same time as the response of this flower bulb to depth of planting and bulb size at planting that is between April and November 2009. This study was conducted in the same manner as that of the hyacinth experiment because the same nutrients were applied at the same rate, to the soil on which the lily bulbs were planted. However, in this case, nutrients application started at 2 weeks after planting the lily bulbs. Results indicated that plants responded to these nutrients generally by producing higher vegetative growth relative to the control. As was observed in the case of hyacinth, vegetative growth increased with increasing level of application of either nutrient, but the application of $(NH_4)_2SO_4$ enhanced the growth of leaves and plant height more than did Na_2HPO_4 (Fig 5.23a, b, c, d), though 60 or 90 mM of the latter also produced significantly higher effects as compared to the control. The trend in chlorophyll content was the same as that of hyacinth because plants that received nutrients through the application of $(NH_4)_2SO_4$ recorded higher chlorophyll content than the control and those to which Na_2HPO_4 was applied. The increased chlorophyll concentration of plants fed with $(NH_4)_2SO_4$ coupled with better growth of leaves of these plants resulted in their having higher photosynthetic rates and stomatal conductance as compared with the control and those fed with Na_2HPO_4 (Fig 5.24a, b and c). However, unlike the case of hyacinth where Na_2HPO_4 enhanced inflorescence development more than $(NH_4)_2SO_4$, in the case of the lily bulb, it was the application of $(NH_4)_2SO_4$ that gave better flower quality (increases in length of florets and peduncle) than Na_2HPO_4 (Fig 5.25), though the application of 90 mM of Na_2HPO_4 outperformed the control in terms of the production of flowers. Also, plants fed with $(NH_4)_2SO_4$ retained their flowers on the plant for a longer time than did those that received application from Na_2HPO_4 and the control. This is because higher percentage of flowers had aborted at 14 weeks after planting (Fig 5.25c) from the control and plants from the Na_2HPO_4 regimes than those that received application from $(NH_4)_2SO_4$. Also plants fed with $(NH_4)_2SO_4$ produced higher gain in weight than the control and those from the Na_2HPO_4 regimes. These plants also had higher number and weight of bulblets as compared to the control and those fed with Na_2HPO_4 (Fig 5.26). As was observed in the case of hyacinth, plants fed with Na_2SO_4 produced similar responses as those of the control (Table 5.4a, b).

(a)



(b)

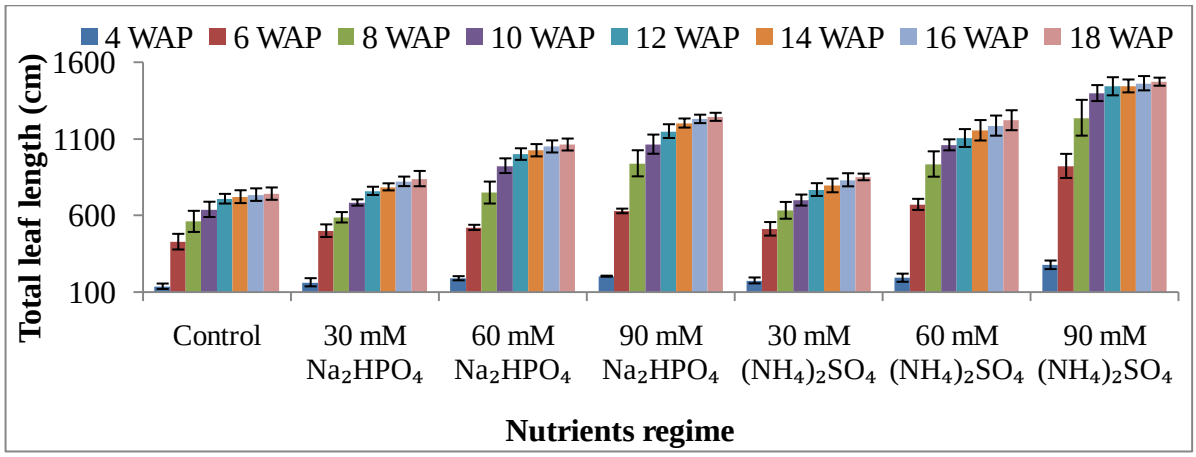
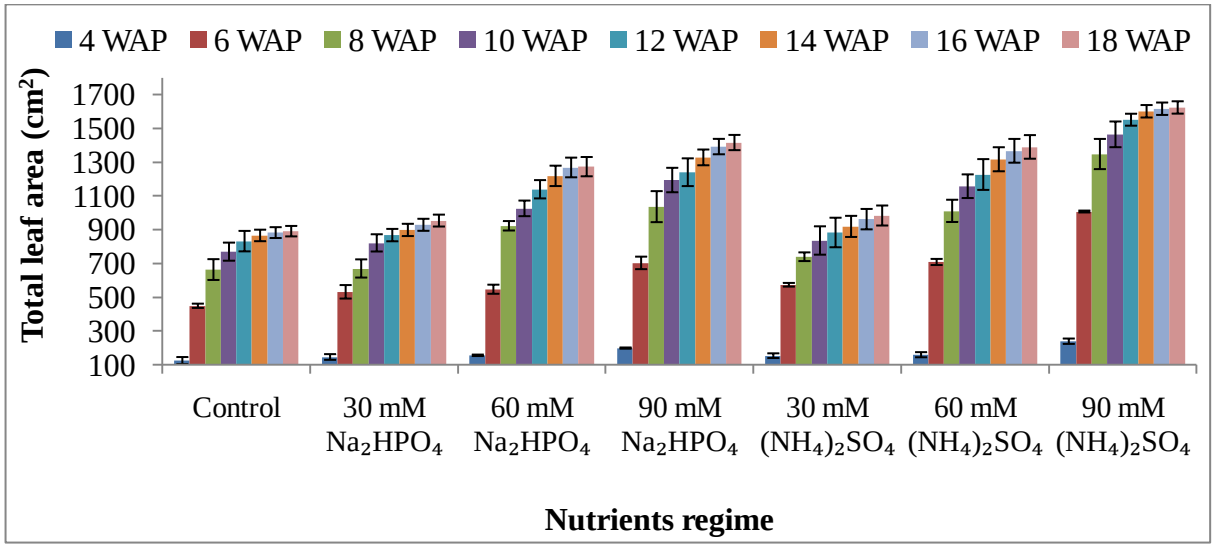


Fig 5.23a: Influence of nutrients feeding on vegetative growth of the lily bulb: (a) total leaf width (b) total leaf length. Bars represent the mean values ± standard error of three plants.

(c)



(d)

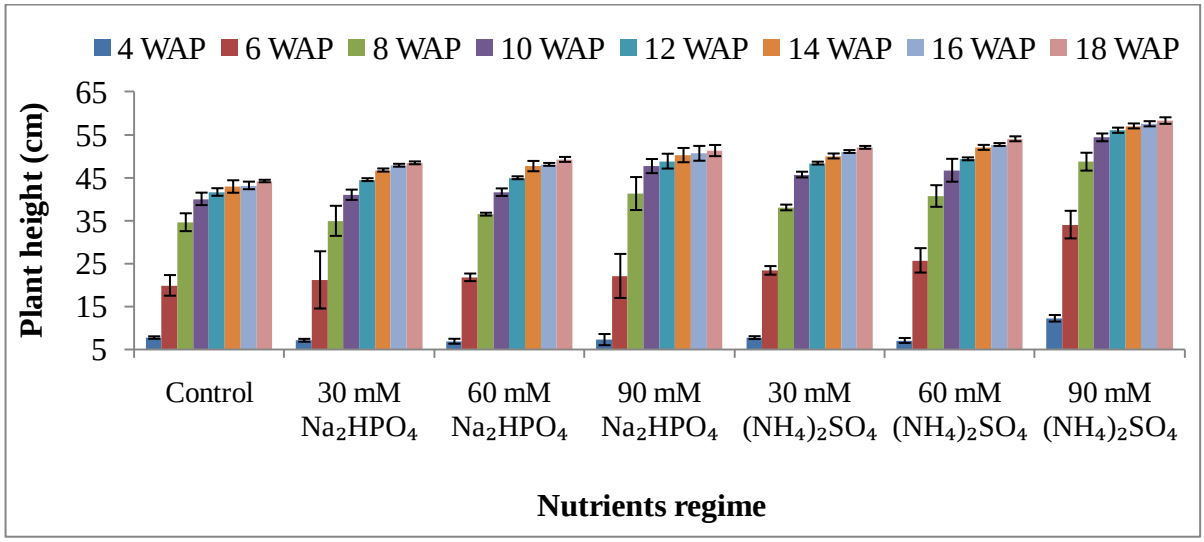
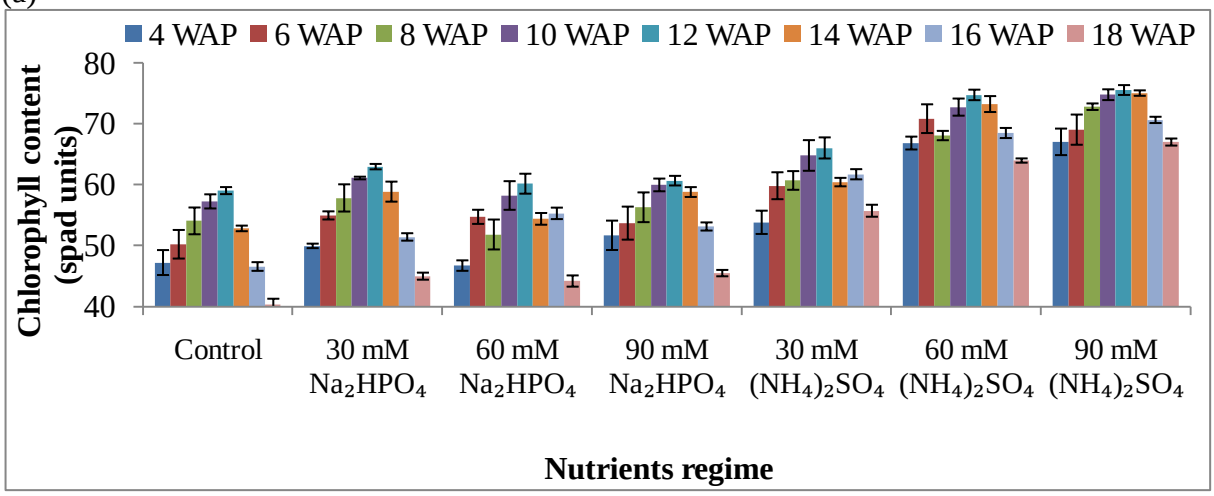


Fig 5.23b: Influence of nutrients feeding on vegetative growth of the lily bulb: (c) total leaf area and (d) plant height. Bars represent the mean values ± standard error of three plants.

(a)



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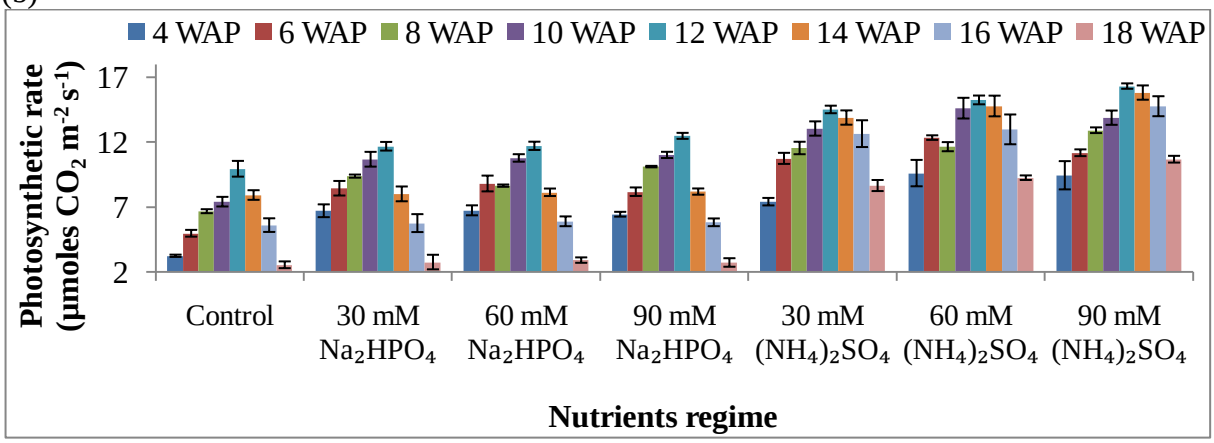

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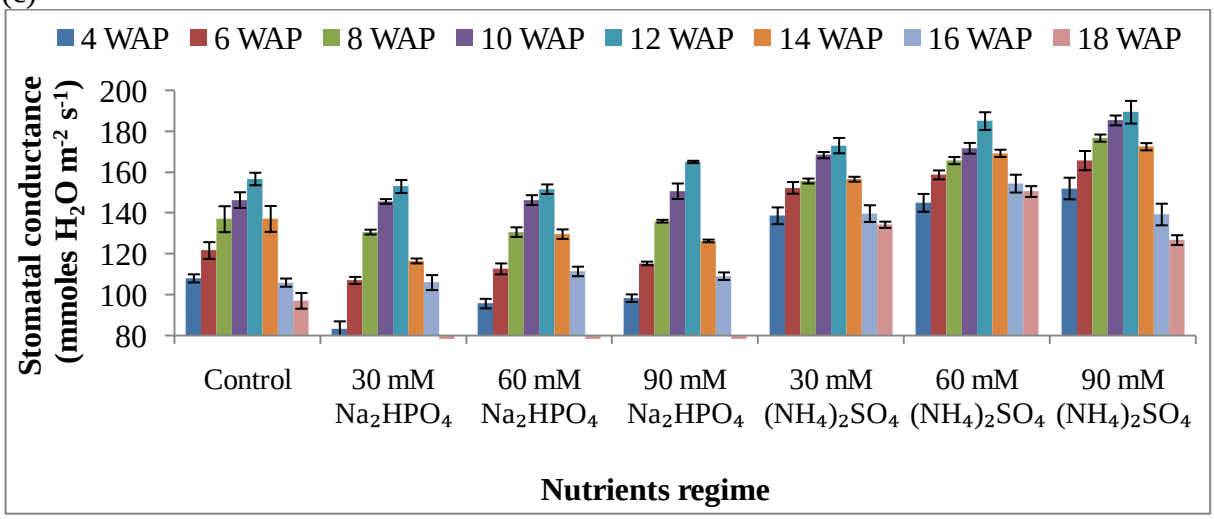
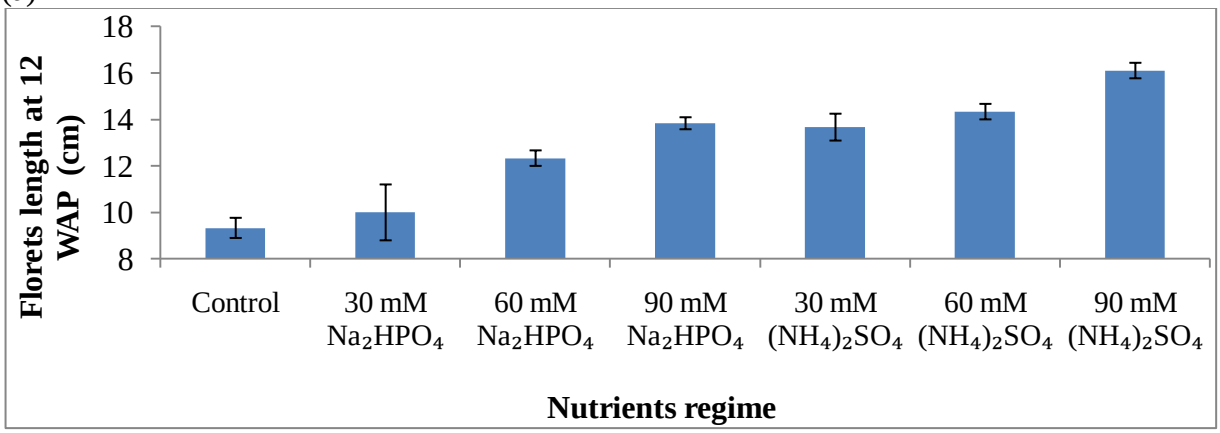


Fig 5.24: Impact of nutrients feeding on (a) chlorophyll content, (b) photosynthetic rate, and (c) stomatal conductance of the lily. Bars represent the mean values ± standard error of three plants.

(a)



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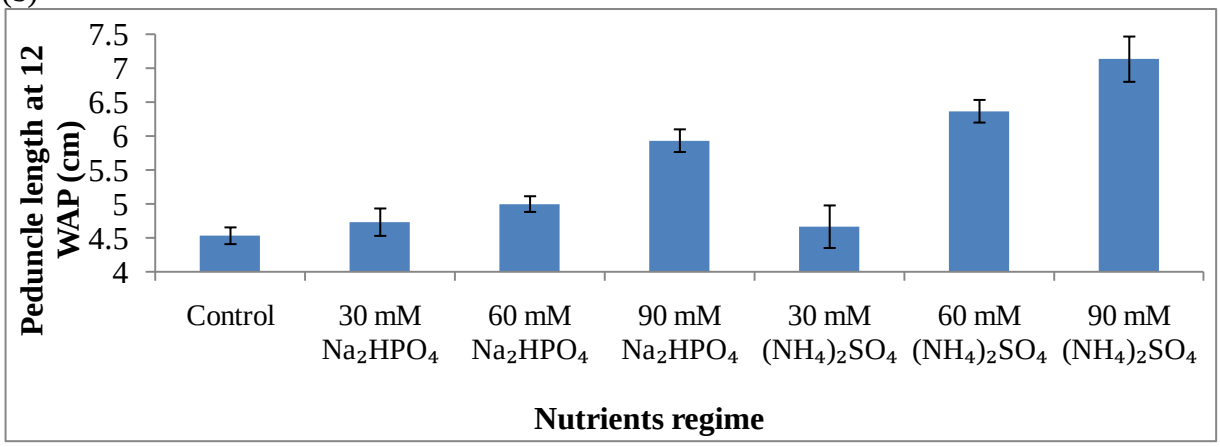


(c)

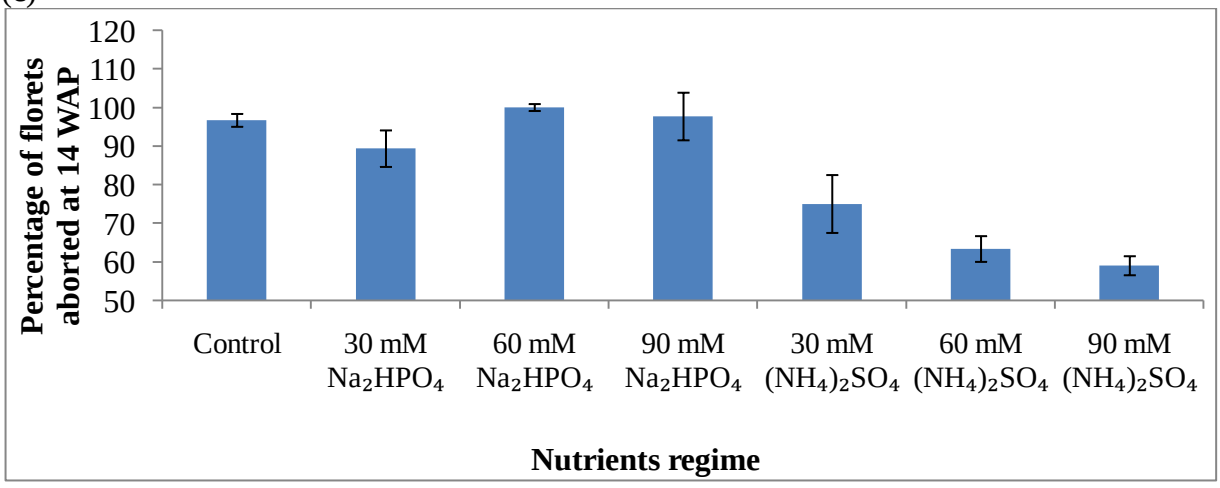
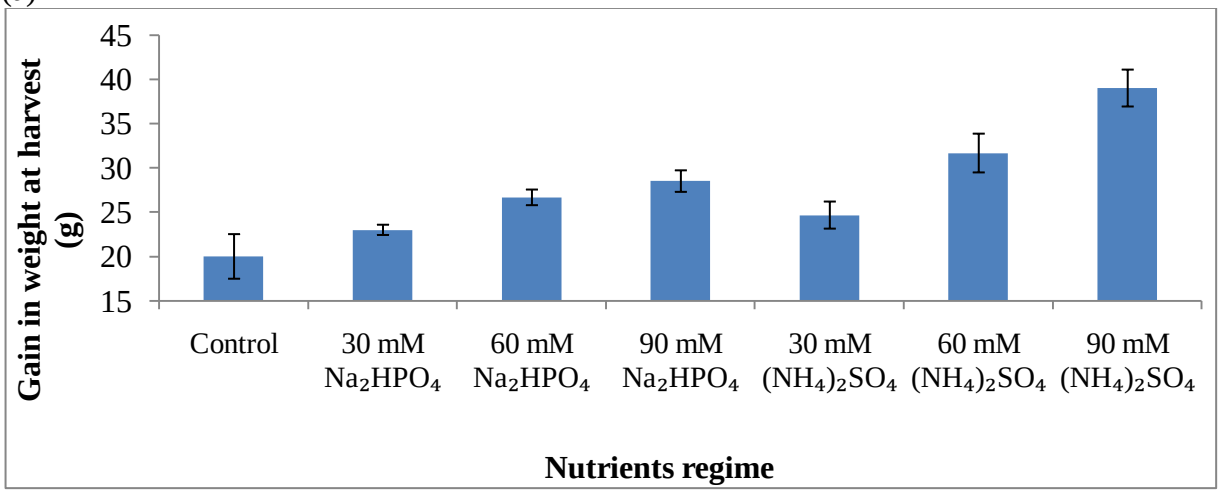


Fig 5.25: Influence of nutrients supply on florets development of the lily bulb, (a) floret length, (b) peduncle length, and (c) percentage of florets that aborted at 14 WAP. Bars represent the mean values ± standard error of three plants.

(a)



(b)

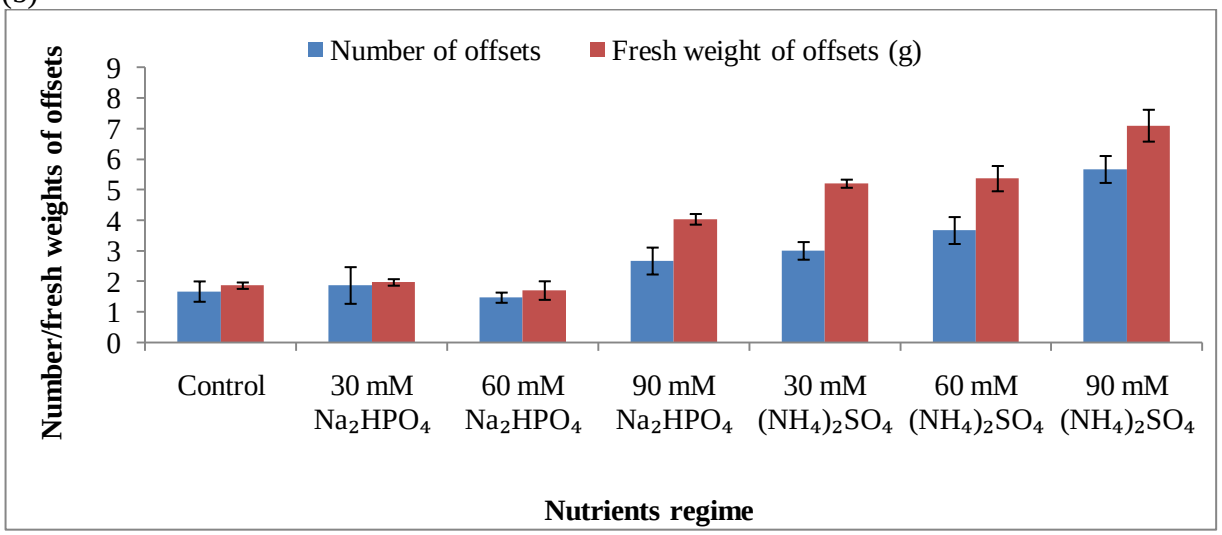


Fig 5.26: Effects of nutrients application on (a) weight gain at harvest of the mother bulb and (b) bulblets formation of the lily. Bars represent the mean values ± standard error of three plants.

Table 5.4: Parameters measured of the lily from the control and the Na_2SO_4 nutrient regime. Results are the mean values ± standard error calculated from three plants.

(a) Total leaf width (cm)

	Weeks after planting							
	4	6	8	10	12	14	16	18
Control	44.7 ± 4.2	71.7 ± 2.9	96.8 ± 5.1	111.6 ± 6.7	124.8 ± 5.1	131.1 ± 6.9	134.45± 4.9	137.76 ± 5.75
30 mM Na_2SO_4	46.2± 5.8	73.5 ±2.7	98.7 ± 9.1	114.9 ± 9.0	128.6 ± 7.3	132.56± 8.7	136.2 ± 6.7	138.85 ± 5.53
60 mM Na_2SO_4	45.6 ±0.8	72.5 ± 3.5	97.3 ± 16.5	112.1 ± 3.3	127.6 ± 6.9	133.0 ± 5.6	135.6 ± 3.6	137.35 ± 6..47
90 mM Na_2SO_4	47.6 ± 1.8	74.4 ± 7.4	99.1 ± 3.3	115.4 ± 7.6	129.8 ± 3.4	135.8 ± 4.5	138.9 ± 2.5	139.02± 2.32

(b) Total leaf length (cm)

Control	136.4± 17.6	427.4± 25.5	559.9 ± 68.9	638.4 ± 49.9	708.0 ± 31.5	721.3±41.9	734.6 ± 40.9	741.2 ± 40.3
30 mM Na_2SO_4	139.5± 10.2	431.5 ± 18.8	564.8 ± 55.7	644.7 ± 54.7	712.8 ± 52.7	723.9 ± 39.5	738.1 ± 37.5	748.6 ± 36.3
60mM Na_2SO_4	137.0± 7.8	429.7 ± 7.2	561.4 ± 34.9	640.2 ± 18.3	709.9 ± 27.4	726.9 ± 38.9	737.0 ± 38.3	745.5 ± 37.9
90 mM Na_2SO_4	140.8 ± 27.1	434.8 ± 27.6	566.7 ± 64.9	649.3 ± 30.1	714.4 ± 42.2	729.6 ± 28.6	742.7± 26.7	752.2± 25.5

(c) Total leaf area (cm^2)

Control	123.9 ± 20.9	448.3 ± 12.7	662.8 ± 61.6	768.9 ± 53.5	831.2 ± 60.6	864.9 ± 34.1	881.8 ± 32.1	890.2 ± 30.9
30 mM Na_2SO_4	125.4 ± 2.9	458.4 ± 13.2	682.9 ± 44.2	773.1 ± 41.1	849.3 ± 63.1	868.8 ± 54.9	888.5 ± 53.9	893.4 ± 53.4
60mM Na_2SO_4	124.5 ± 10.3	450.9 ± 35.4	673.3 ± 46.8	770.9 ± 43.9	839.2 ± 39.9	866.3 ± 40.4	893.9 ± 39.7	904.7 ± 39.3
90 mM Na_2SO_4	126.8 ± 18.5	457.1 ± 18.3	682.7 ± 42.8	781.1± 55.0	845.8 ± 32.5	871.6 ± 47.8	895.6 ± 45.8	910.5± 44.6

(d) Plant height (cm)

Control	8 .0± 0.3	20.6± 2.4	36 ± 2.1	41.6 ± 1.5	44.3 ± 0.8	44.6 ± 1.4	44.9 ± 0.9	45 .0± 0.3
30 mM Na_2SO_4	7.5 ± 0.6	20.3 ± 4.6	35.73 ± 4.9	41.0 ± 3.2	43.3 ± 3.2	44.5 ± 3.2	44.7 ± 3.2	45 .0 ± 0.6
60mM Na_2SO_4	7 .0± 0.6	18.3 ± 3.2	35.33 ± 2.9	40.6 ± 1.7	42.0 ± 2.1	42.3 ± 2.9	42.5 ± 2.1	43.0 ± 0.6
90 mM Na_2SO_4	7.3 ± 0.3	18.6 ± 3.3	35.5 ± 4.2	41.0 ± 4.0	43.0 ± 3.5	43.5 ± 3.4	43.8 ± 3.5	44 .0 ± 0.3

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(e) Chlorophyll content (spad units)

Control	47.2 ± 2.1	50.2 ± 2.3	54.0 ± 2.2	57.2 ± 1.2	59.0 ± 0.6	52.8 ± 0.5	46.5 ± 0.7	40.3 ± 0.96
30 mM Na_2SO_4	46.9 ± 0.7	51.9 ± 1.1	53.9 ± 1.9	56.1 ± 2.5	57.4 ± 2.7	52.4 ± 0.5	46.7 ± 0.7	41.0 ± 0.98
60mM Na_2SO_4	49.1 ± 2.1	54.1 ± 2.4	54.7 ± 2.3	56.4 ± 0.7	56.5 ± 1.2	53.3 ± 0.7	48.3 ± 0.7	43.2 ± 0.78
90 mM Na_2SO_4	48.5 ± 3.2	51.5 ± 3.5	53.4 ± 2.9	55.1 ± 1.1	56.3 ± 0.2	53.5 ± 0.5	48.2± 0.8	42.8± 1.2

(f) Photosynthetic rate (µmoles CO_2 m^{-2} s^{-1})

Control	3.2 ± 0.1	4.9 ± 0.3	6.6 ± 0.2	7.4 ± 0.4	9.9 ± 0.6	7.9 ± 0.3	5.5 ± 0.5	2.5 ± 0.3
30 mM Na_2SO_4	3.0 ± 0.1	4.8 ± 0.2	6.5 ± 0.3	7.5 ± 0.6	9.7 ± 0.3	7.7 ± 0.5	5.4 ± 0.8	2.4 ± 0.2
60mM Na_2SO_4	3.1 ± 0.5	4.8 ± 0.5	6.4 ± 0.2	7.4 ± 0.5	9.2 ± 0.3	7.8 ± 0.5	5.6 ± 0.6	2.6 ± 0.5
90 mM Na_2SO_4	2.9 ± 0.2	4.6 ± 0.4	6.4 ± 0.1	7.6 ± 0.5	9.2 ± 0.3	7.7 ± 0.5	5.5 ± 0.5	2.5 ± 0.4

(g) Stomatal conductance (mmoles H_2O m^{-2} s^{-1})

Control	118.0 ± 2.0	131.6 ± 4.1	132.0 ± 6.3	146.3 ± 3.8	166.6 ± 3.1	137.1 ± 6.3	105.9 ± 2.0	97.0 ± 3.8
30 mM Na_2SO_4	120.0 ± 4.9	133.6 ± 4.8	136.0 ± 1.5	158.6 ± 6.9	164.3 ± 0.6	133.2 ± 1.4	113.6 ± 4.9	105.9 ± 6.9
60mM Na_2SO_4	122.3 ± 4.8	136.0 ± 3.5	133.3 ± 0.8	157.6 ± 1.5	162.0 ± 2.7	130.4 ± 0.8	110.7 ± 4.8	103.0 ± 1.5
90 mM Na_2SO_4	121.6 ± 1.6	135.3 ± 3.9	135.6 ± 0.6	158.0 ± 4.1	163.3 ± 1.2	133.1 ± 0.6	113.7 ± 1.6	106.1± 4.1

(h) Other parameters

	Floret length (cm)	Peduncle length (cm)	florets abortion (%)	Gain in weight (g)	Number of offsets	Fresh weight of offsets (g)
Control	9.3 ± 0.3	4.5 ± 0.6	96.6 ± 15.3	20.0 ± 2.5	1.6 ± 0.3	1.8 ± 0.1
30 mM Na_2SO_4	9.0 ± 1.2	4.6 ± 0.4	81.0 ± 10.1	18.6 ± 3.3	1.6 ± 0.4	1.8 ± 0.2
60mM Na_2SO_4	9.4 ± 1.2	4.5 ± 0.8	83.3 ± 8.3	20.3 ± 1.2	1.3± 0.4	1.6 ± 0.2
90 mM Na_2SO_4	9.2 ± 0.6	4.6 ± 0.3	72.3 ± 7.3	19.0 ± 1.5	1.6 ± 0.3	1.7 ± 0.2

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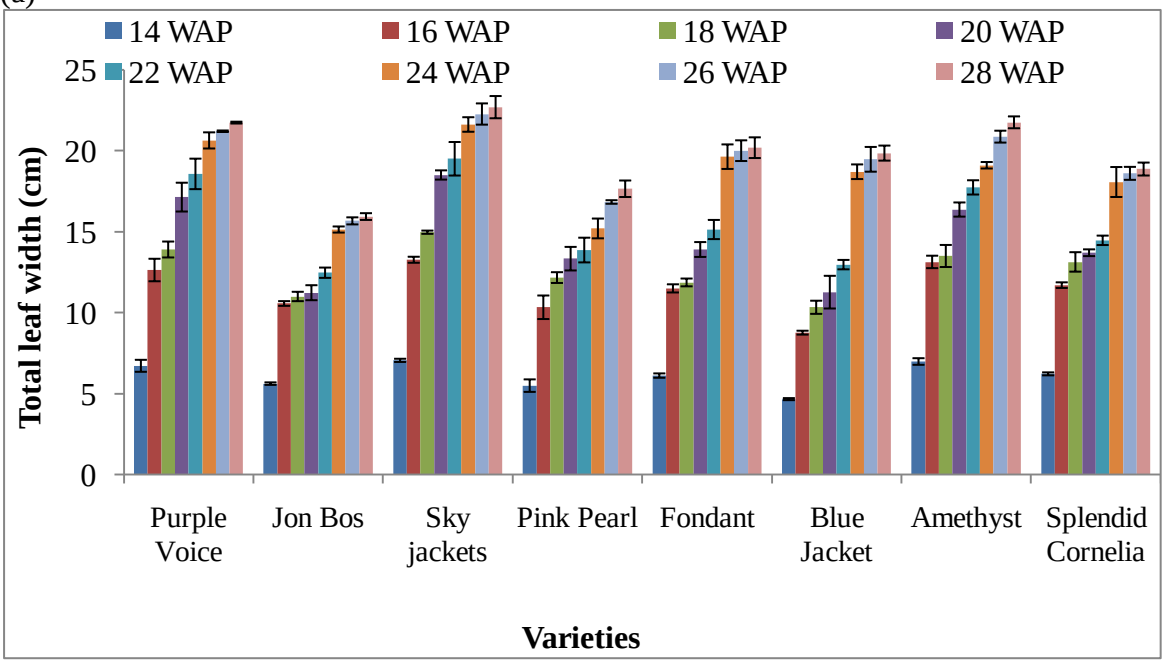
5.2.4 Influence of varieties on growth and development

Studies on effects of varieties on bulb growth and development were conducted using hyacinth bulbs. The experiments were carried out between September 2009 and April 2010. In all, eight varieties of hyacinth namely Purple Voice, Jon Bos, Sky Jackets, Pink Pearl, Fondant, Blue Jacket, Amethyst and Splendid Cornelia were evaluated to determine their characteristics in terms of bulb yield or flower production. Results indicated that all the varieties except Jon Bos and Pink Pearl produced relatively high vegetative growth as shown by their leaf width, length and area (Fig 5.27a and b; Fig 5.28a). Sky Jacket produced the highest leaf growth whilst Jon Bos recorded the least leaf growth. In general, rate of leaf growth was low from 14 to 22 weeks after planting but high between 22 and 24 weeks after planting. Chlorophyll content also increased from 14 to 24 weeks after planting and decreased from 24 to 28 weeks after planting. Sky Jacket, followed by Purple Voice gave the highest values in terms of chlorophyll concentration (Fig 5.28b) whilst Jon Bos and Blue Jackets recorded the least values. With regard to flower (quality), Fondant produced the highest inflorescence height, inflorescence length, inflorescence diameter, inflorescence stalk diameter and number of florets, followed by Blue Jacket and Sky Jacket in that order, whilst Pink Pearl and Amethyst recorded the least of these parameters (Fig 5.29a and b; Fig 5.30a and b; Fig 5.31a). The inflorescence of all varieties opened at or before 24 weeks after planting (Fig 5.32) with the exception of Amethyst whose florets were fully opened at 26 weeks after planting. Trend in weight gain at harvest closely followed that of leaf growth because Sky Jacket, followed by Purple Voice, produced the highest weight gain whilst Jon Bos recorded the lowest gain in weight at harvest (Fig 5.31b). A similar observation was made in terms of number and weight of offsets produced by these varieties.

(a)



(b)

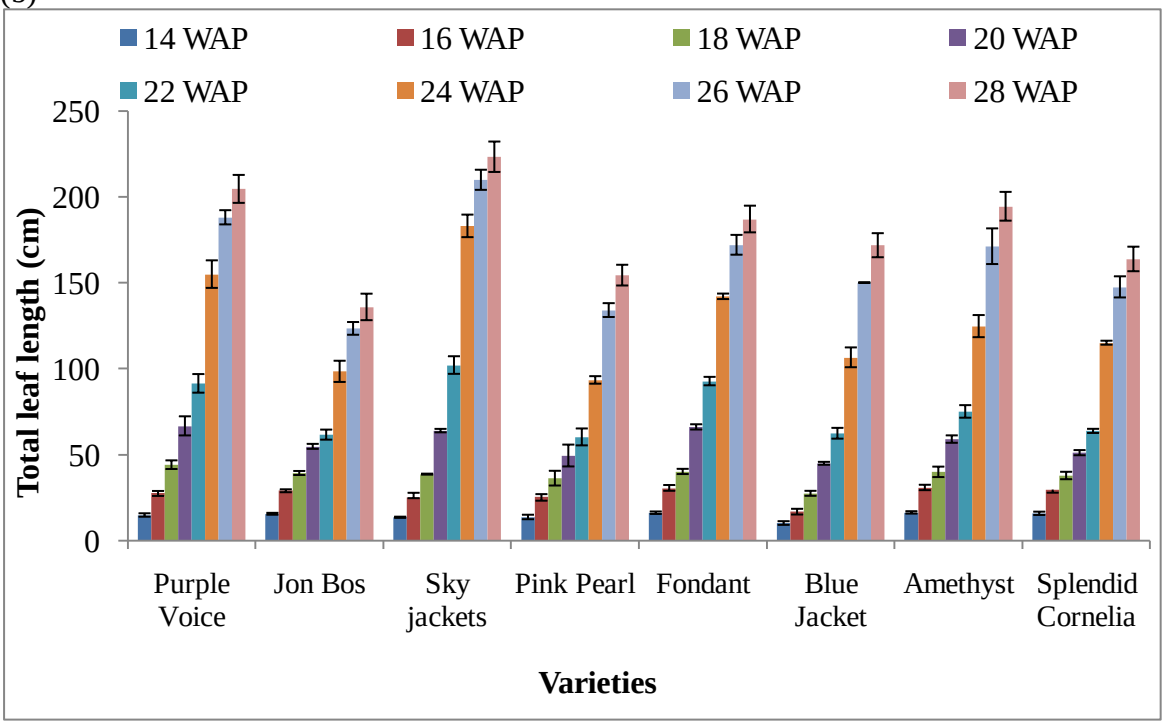
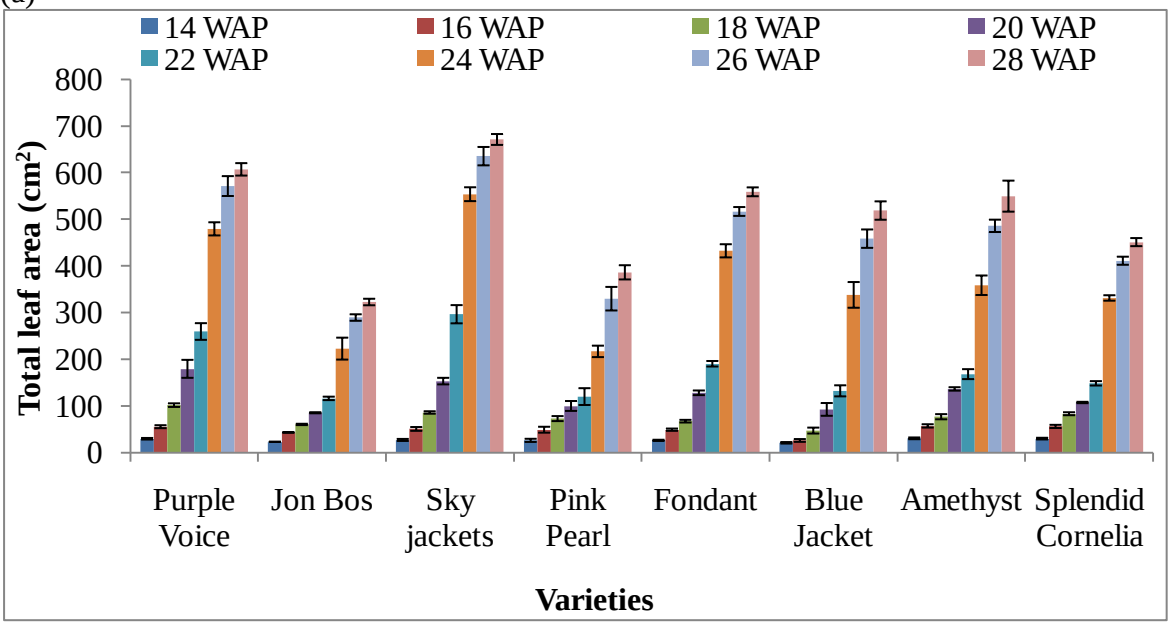


Fig 5.27: Variations in leaf growth of the eight varieties: (a) total leaf width, and (b) total leaf length of hyacinth. Bars represent the mean values ± standard error of five plants.

(a)



(b)

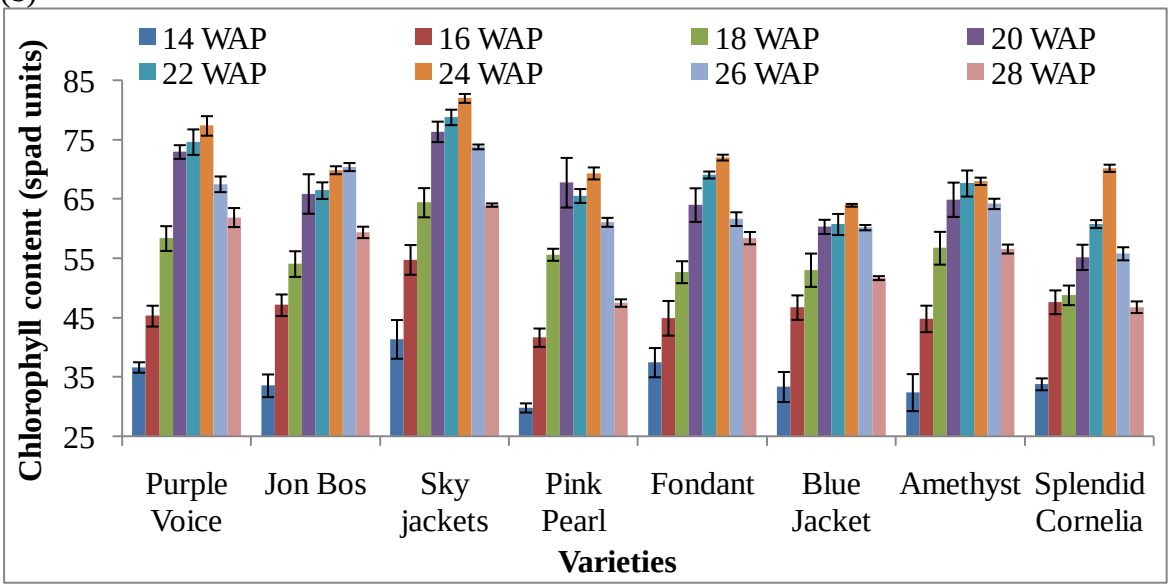
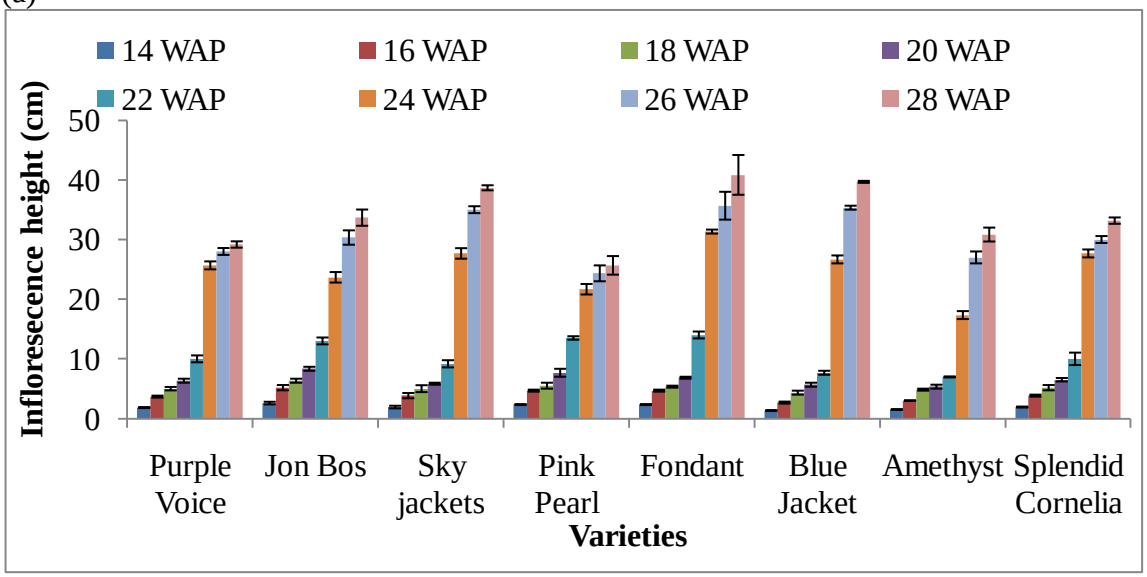


Fig 5.28: Influence of variety on (a) total leaf area, and (b) chlorophyll content of hyacinth. Bars represent the mean values ± standard error of five plants.

(a)



(b)

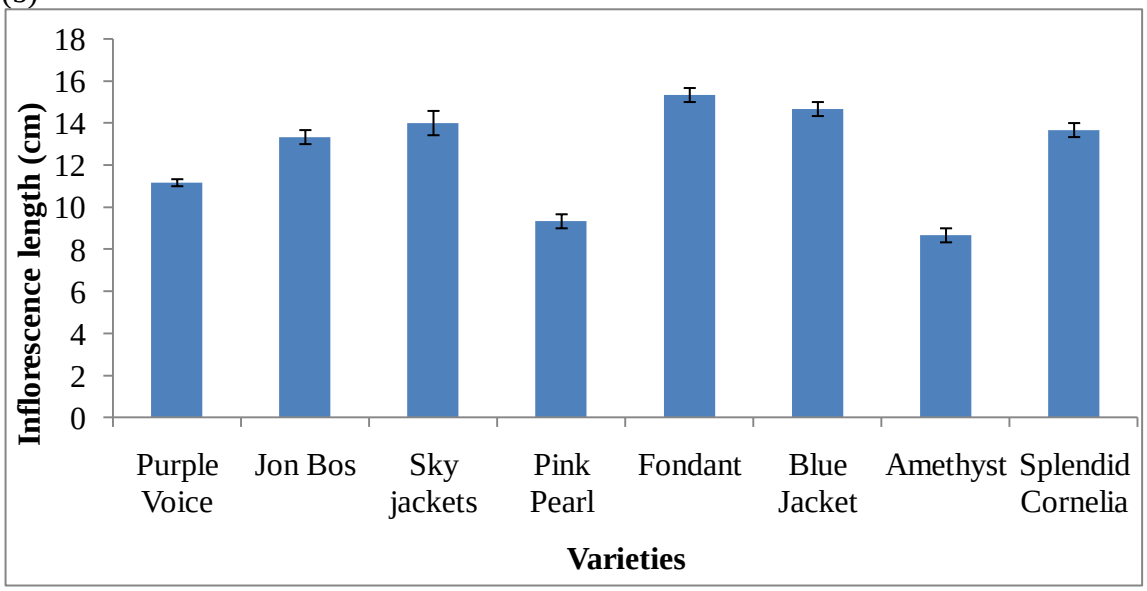
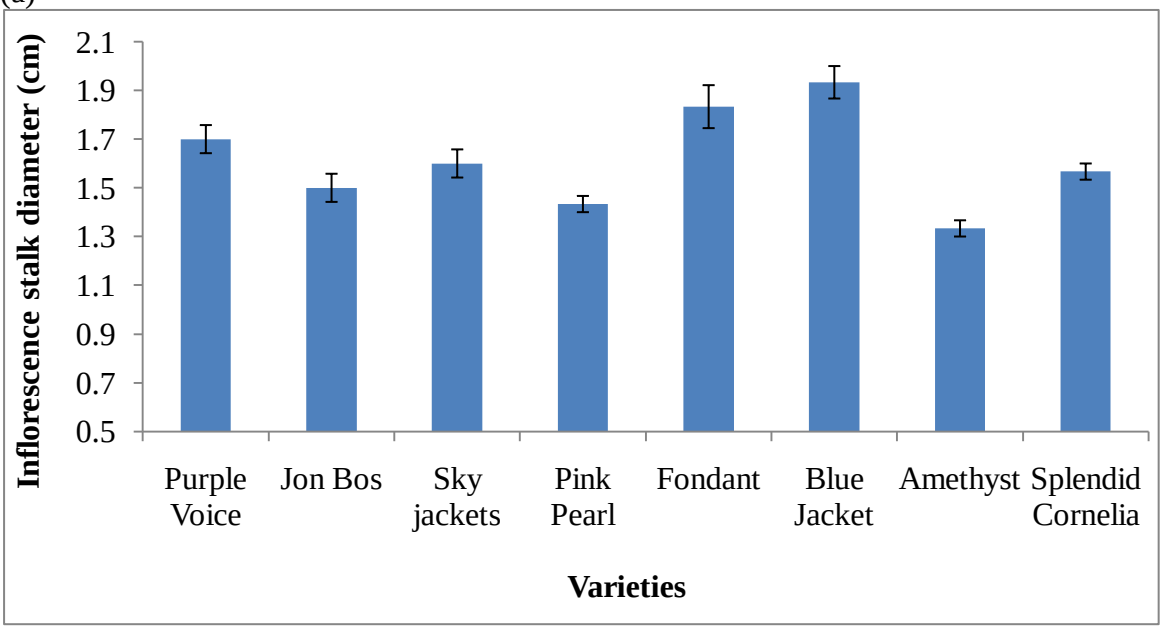


Fig 5.29: Influence of varieties on (a) inflorescence height, and (b) inflorescence length of hyacinth. The inflorescence lengths presented are the values measured at 28 weeks after planting. Bars represent the mean values ± standard error of five plants.

(a)



(b)

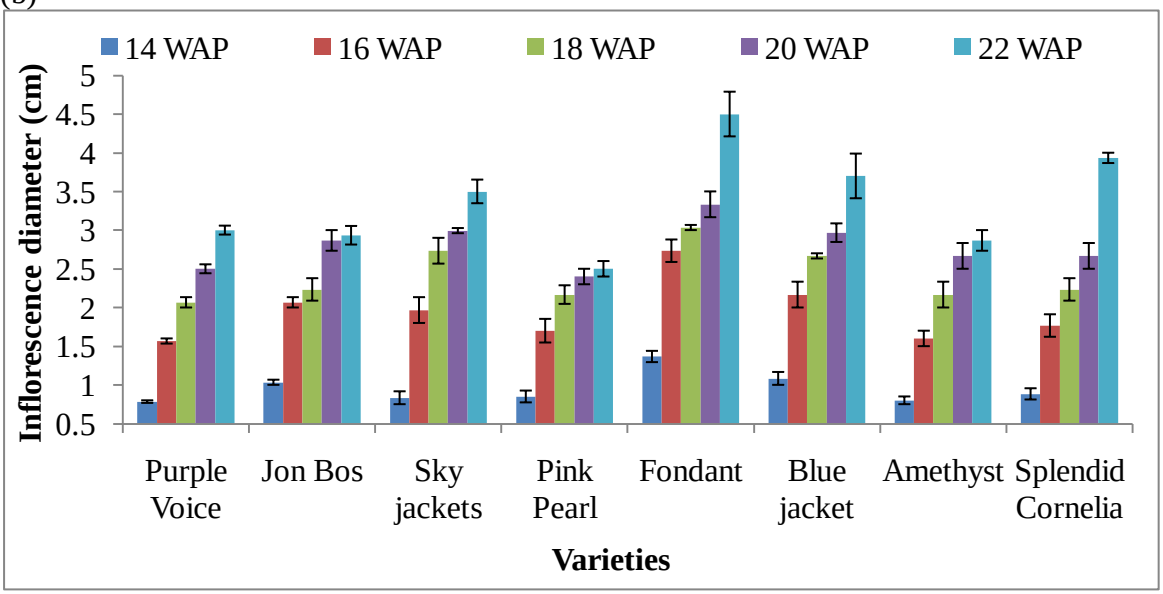
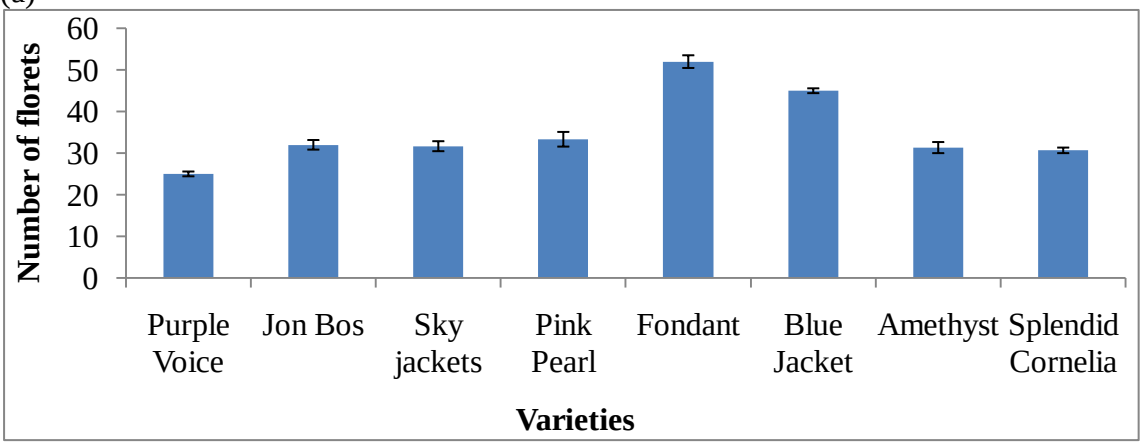
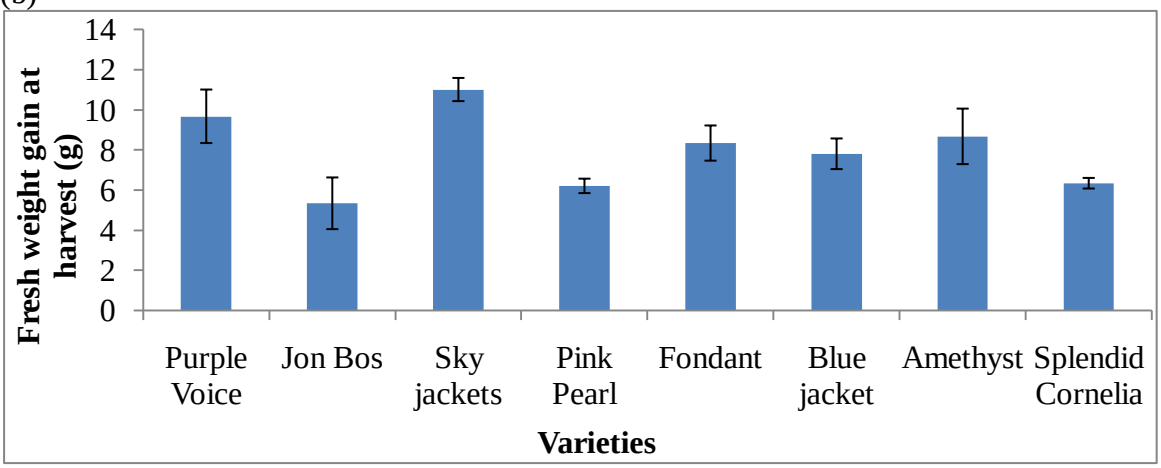


Fig 5.30: Influence of varieties on (a) inflorescence stalk diameter, and (b) inflorescence diameter of hyacinth. Bars represent the mean values ± standard error of five replicates.

(a)



(b)



(c)

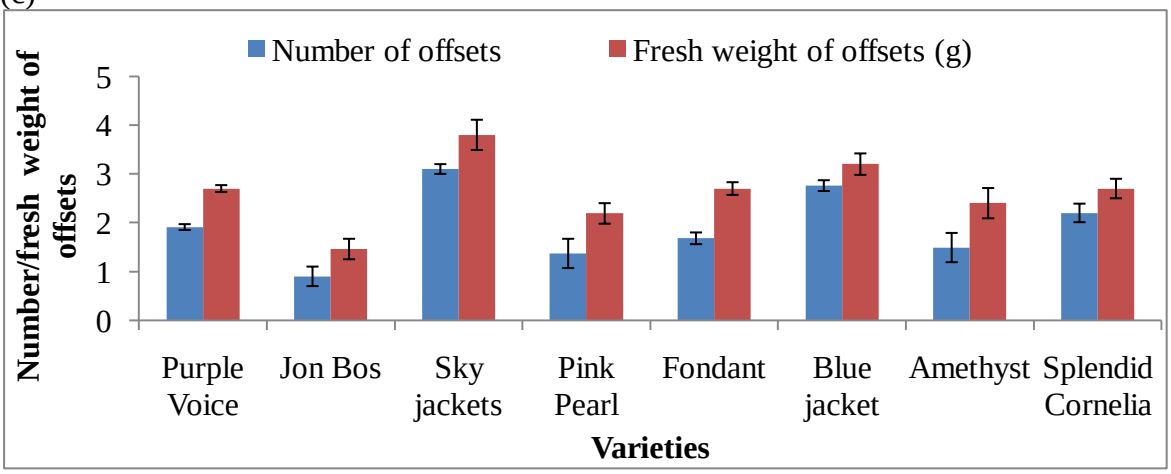


Fig 5.31: Influence of varieties on (a) number of florets, (b) fresh weight gain of the mother bulb, and (c) offsets formation of eight varieties of hyacinth during the 2009/2010 season. Bars represent the mean values ± standard error of five replicates.

(a) (b)

(c) (d)

Fig 5.32: Photographs of the eight varieties of hyacinth. Pictures of these varieties were taken at 24 weeks after planting: (a) Purple voice (left) and Pink pearl (right), (b) Jon bos (left) and Sky jacket (right), (c) Fondant (left) and Blue jacket (right) and (d) Splendid cornelia (left) and Amethyst (right).

5.3 Discussion

5.3.1 Bulbs size at planting

Studies on the effects of bulb size at planting on growth and development in hyacinth as well as the lily bulb demonstrated that the size of the bulb used for planting is very important in the selection of planting materials for bulb production. This is because bulb size influenced vegetative growth both in the hyacinth and the lily. In general, as the size of the planted bulb increased, vegetative growth (leaf length, width, area and plant height) also increased. A similar observation was made by Rees (1969) in tulips and Burton (1966) in potatoes. The increase in magnitude of these parameters in the present study in proportion to the bulb size used for planting may be attributed to the amount of reserve stored in the bulb prior to planting. Chapter 3 highlighted the role of reserve carbohydrates of bulbous plants in their growth and development. That is, the initial growth of geophytes mainly depends on reserve materials stored in the bulb scales because these plants store and remobilise the reserved metabolites, mainly carbohydrates for their own growth and development. In general, since large bulbs are expected to have larger amounts of carbohydrates and other reserves than smaller bulbs, the former should therefore have better growth and development than the latter. Of course, the smallest bulb sizes behaved just like the peeled bulbs (Chapter 4) whose bulb reserves were reduced as a result of scale removal with the result that their growth and development was adversely affected. My results presented here, and those from Chapter 4 have emphasized that reserve carbohydrates play a crucial role in the growth and development of bulbs. It also means that the performance of geophytes and other plants propagated by vegetative reproduction may be influenced by the amount of stored reserves present at the time of planting (Bremner and El Saeed, 1963; Burton, 1966). However, contrary to Rees (1969) observation that small bulbs grew faster than big bulbs in tulips, results from the present study revealed that in either hyacinth or lilies, large bulbs recorded higher relative growth rate than small bulbs. Hidekazu *et* al. (1998) also observed that when larger tuber fragment is used as seed yam for propagation, the initial growth rate was higher than when small tuber fragment was used for yam production. The observed increase in relative growth rate of the large bulbs as compared with the small ones, according to Hidekazu *et* al. (1998), is because in vegetatively propagated plants, large planting materials are characterised by higher and more conspicuous reduction in weight during sprouting than small bulbs and the higher

the reduction in weight during sprouting, the higher the growth rate and this is what was observed in this study.

In both hyacinth and the lily, chlorophyll content of plants produced from the large bulbs was generally higher than those obtained from the small bulbs. In hyacinth, plants from the small bulbs (10-30 g), maintained values of chlorophyll content from 12 to 22 weeks after planting whilst the plants from medium bulb (40-50 g) and the large bulbs (60-80 g) recorded a drop in this parameter at 18 weeks after planting. Thus in both hyacinth and the lily, the drop in chlorophyll concentration of plants produced from the large bulbs was higher than the medium bulbs; whilst the small bulbs maintained their chlorophyll concentration during the time that this parameter was measured. The reduction in chlorophyll content of plants from the large and medium bulbs after 18 weeks of growth as compared to those of the small ones, among other factors would be attributed to the natural process of ageing that occurred earlier in the case of plants produced from the large and medium bulbs, than plants obtained from the small bulbs. In general, the importance of chlorophyll in plants is mainly to absorb the light energy without which photosynthesis cannot proceed. It means that the amount of this pigment found in the plant is a measure of rate of photosynthesis and a deficiency or a drop in chlorophyll content of plants may adversely affect the rate of photosynthesis. It implies that plants from small bulbs would have the ability to maintain their photosynthetic rate for a longer time than plants produced from large bulbs. Apart from influencing the rate of photosynthesis, chlorophyll content of plants is an indicator of plant vigour. Therefore, a low chlorophyll content of plants may be as a result of a stress resulting from drought, disease infestation or mineral deficiency. In addition, a low chlorophyll concentration of plant may imply that the natural process of ageing (senescence) in plants is occurring. Since bulbs of different sizes were planted on the same type of soil, and no diseases were detected during the growth of the bulbs, and also no stresses were imposed on the plants, a reduction in chlorophyll of any of the treatments (plants produced from the different bulb sizes at planting) was assumed to be the result of senescence, that is, the natural process of ageing of the plants. During senescence, the rate of photosynthesis reduces and this was the behaviour of plants produced from large and medium bulbs. Hyacinth is a spring flowering bulb whilst lily is a summer bulb, and in this study, these two groups of bulbs shared similar characteristics in terms of vegetative growth and chlorophyll development, in relationship with the size of the

planted bulbs. The above observations imply that a plant produced from large bulb grows faster to complete their life cycle but ages more quickly than that from small bulbs. Also during senescence, not only does chlorophyll concentration decrease but in general, photosynthetic rate as well as stomatal conductance of the plant also reduces. In hyacinth, photosynthetic rate and stomatal conductance of plants produced from large and small bulbs were not measured due to equipment breakdown and failure at the time of data collection. But in the lily, measurements of chlorophyll, photosynthetic rate and stomatal conductance showed that reduction in these parameters especially towards the end of the season was higher in plants from the large bulbs than those from the small bulbs. The higher percentage of florets aborted in the case of plants produced from the large bulbs compared with the proportion of florets aborted from plants produced from small bulbs in the lily is also an indication that plants produced from large bulbs mature quickly and reach senescence in flowering bulbs than those obtained from small bulbs since senescence is also characterised by flower abortion, among other factors.

The study also revealed that in both hyacinth and the lily, flower quality (inflorescence height, length, diameter, and number of florets, florets length and peduncle length) increased with increasing bulb size. This agrees with the observation made by De Munk and Schipper (1993) who also worked on Iris bulb and reported that weight was an important indicator for flowering in that species. Flowers, florets or inflorescence are important sink organs in flowering bulb that depend on the reserves stored in the bulb for their initial growth and development. Results of Chapter 4 revealed the importance of bulb scales particularly the outermost scale to flower quality in both hyacinth and the lily because the removal of the scales affected flower production. The explanation that large bulbs have higher reserves than small bulbs and therefore are expected to support the growth and development of bulbs is also responsible for the fact that large bulbs have better flower quality than small bulbs and this also explains why higher fresh weight of the mother bulb or bulblets were recorded of large bulbs as compared to the small sized bulbs. Results of the hyacinth experiment indicated that, the possession of a large bulb has little advantage, overall, to the plant in terms of vegetative growth and flower production. This is because after the 50 g bulb size, the results look markedly similar. It means therefore that the extra carbohydrate reserves of plants produced from large bulbs (> 50 g bulb sizes) were not used for vegetative growth. Maybe, this extra

carbohydrate might be useful later during the life cycle of the bulb for survival during unfavourable environmental conditions.

In conclusion, it would be difficult to make a recommendation as to what bulb size is desirable during the planting of hyacinth or the lily unless the needs of the consumer or the aims of the grower are taken into consideration. This is because plants from large bulbs (50-80 g of hyacinth and 30-49 g in lily) generally grow well and produce better flower quality than those from small bulbs. Also, plants from large bulbs generally produce large bulbs at harvest and they also have better offsets formation as compared to small bulbs. However, any bulb size, particularly in the case of hyacinth, above 50 g will not give any results better than the 50 g bulb size in terms of vegetative growth and flower quality. Small bulbs retain chlorophyll and photosynthetic rate longer than large bulbs. Also, when weight gain is computed in terms of percentages, small bulbs produce higher weight gain at harvest than large ones. Thus for vegetative biomass and flower production or bulblets formation, growers should use bulbs that are not >60 g (hyacinth) and > 30 g (lily) as planting material. On the contrary, if the grower is interested in bulb yield (gain in fresh weight at harvest) and not vegetative biomass, flowers or bulblets, then any bulb size that is <40 g (hyacinth) or < 30 g (lily) could be used for planting since these sizes will give high percentage gain in weight at harvest.

5.3.2 Depth of planting

Investigation into the influence of planting depth on the development of the two species of flower bulbs under consideration revealed that significant variability existed among the parameters measured for the various regimes of planting depths. In the case of hyacinth, bulbs planted at 5 cm depth sprouted earlier than those placed at the soil surface (the 0 cm depth). The observed delay in emergence of the bulbs planted at 0 cm depth as compared to those planted at 5 cm depth is due to the exposure of the former to the air that facilitated moisture loss and prolonged the time for root development or reduced the rate of root formation. After planting, bulbs from all planting regimes apart from those planted at 0 cm depth were covered with soil. In general, soil is a good medium for germination and growth of plants and so the exposure of the bulbs (those planted at 0 cm depth) to the atmosphere, apart from enhancing evaporation of water from the bulbs, denied these bulbs the medium needed for sprouting. A similar

observation was made when carbohydrate metabolism of bulbs planted in soil and those in glass tubes were compared (Chapter 3). In that case, bulbs planted in soil developed roots and shoots earlier and lost reserve carbohydrates (starch) more than those planted in glass tubes. In the planting depth experiment, the bulbs placed at soil surface (0 cm depth) behaved similarly to those planted in glass tubes in Chapter 3. However, the lily bulbs that were placed on soil surface (0 cm depth) did not sprout; neither did they survive.

Depth of planting generally had a detrimental effect on emergence. For the 0, 5, 10, 15 and 20 cm depth, the deeper the depth of planting, the longer it took the bulbs to emerge from the soil after planting. Bulbs planted were of the same cultivar and sprouting might have occurred at the same time but those planted deep had to travel longer distances, pushing the bulbs through the soil before finally emerging out. In general, the delay in emergence of bulbs planted deep (15 cm and 20 cm depth regimes) also resulted in reduced characteristics of vegetative parameters, poor flower quality and reduction in fresh weight gain at harvest. This agrees with Vogel (1963) and Stickler (1962) observation that planting depth is an important agronomic factor that affects growth, development and yields of plants. The observed decrease in vegetative parameters, flower quality and bulb yield at harvest as depth of planting increased could be the result of two causes; the first being the direct effect of planting depth itself and the second one being an indirect effect. That is, plants from deep planting (15 cm and 20 cm depths) recorded lower values of these parameters because they emerged later than those from shallow planting. In hyacinth, whilst plants from the 5 cm depth emerged on average at 35 days after planting, those planted at the depths of 15 cm and 20 cm took on average 120 and 140 days, respectively, to emerge from the soil. That is, the bulbs from the deepest planting depth (20 cm depth) delayed in emergence by 105 days on average relative to those planted at 5 cm depth. Thus in hyacinth, leaves had already emerged and were growing by 14 weeks after planting for bulbs planted at 0, 5 or 10 cm depth (shallow planting regimes) but visible leaves appeared only at 18 weeks after planting for plants belonging to the 15 cm or 20 cm depths (deep planting regimes). Consequently, at 20 weeks after planting, total leaf area values for hyacinth were, 190.44 cm^2, 203.23 cm^2, 113.64 cm^2, 61.97 cm^2 and 14.12 cm^2 for the 0, 5, 10, 15 and 20 cm depths, respectively, whilst at 14 weeks after planting, values for this parameter were 27.61 cm^2, 22.11 cm^2, 0.00 cm^2, 0.00 cm^2 and 0.00 cm^2, respectively, giving

relative growth rates of 3.88 cm day^{-1}, 4.31 cm day^{-1}, 2.71 cm day^{-1}, 1.48 cm day^{-1} and 0.34 cm day^{-1}, respectively. This implies that growth was initially lower in the case of plants from deep planting regimes (15 cm and 20 cm depths) than those from shallow planting. Bulbs planted shallow (0-10 cm depth) therefore produced plants that had competitive advantage in terms of acquisition of resources for growth relative to those from the deep planting regimes. Bulbs planted deep also directed all their resources and energy to pushing their shoot upwards to emerge from the soil before growth and other developments continued. An increase in planting depth therefore prolonged the time from planting to emergence. Notwithstanding, plants were all of the same cultivar and must enter the rest period at the end of the growth season almost at the same time irrespective of planting depth and therefore in deep planting, the growing season was shorter than in shallow planting (Hagiladi *et al,* 1992).

In terms of chlorophyll formation, plants produced from bulbs that were planted deep (15 and 20 cm depths) suffered only at the initial stages of growth because these plants recovered in chlorophyll content towards the end of the season in both hyacinth and the lily. But in the case of hyacinth, the chlorophyll content of the deepest planting regime (20 cm depth) exceeded those of the other regimes towards the end of the season. Thus it looks like, hyacinth plants from the 20 cm depth behaved just like the small bulbs (< 30 g) in terms of senescence, the natural process of ageing in plants. The higher chlorophyll content of the hyacinth plants belonging to the 20 cm depth regime at 28 weeks after planting compared to those from the other planting depth regimes may be an indication of delayed senescence in the former. Senescence is manifested in terms of a reduction in chlorophyll concentration, abscission of leaves, flowers and fruits, leaf die back, among others. This observation is supported by higher percentage of florets abortion that characterised shallow planting in the case of the lily as compared to those of deep planting. The photographs taken of hyacinth plants planted deep (20 cm) at the time of harvesting also appeared green as compared to those from the other planting regimes. All these are evidences that show that in flower bulbs shallow planting is characterised by a hastening in rate of senescence, whilst deep planting (20 cm depth) increased the length of the life cycle such that senescence is delayed. Of course, this is obvious also from the computation of relative growth rates (see above) of plants from the various planting regimes.

At harvest, the hyacinth bulbs were found at depths of 2.83, 7.33, 12.1, 16.9 and 20.86 cm, respectively, for the 0, 5, 10, 15 and 20 cm planting depths and these represent a downward movement of 2.83, 2.33, 2.10, 1.90 and 0.86 cm, respectively. In the case of the lily, bulbs planted at 5, 10, 15 and 20 cm were harvested at depths of 12, 15, 19 and 21 cm representing a downward movement of 7, 5, 4 and 1 cm respectively. This observation demonstrates that bulbs generally make a downward movement in the soil when planted in the soil. This is in agreement with the statement made by Galil (1961). According to his research, geophytic plants in their natural habitat struggle in the soil to maintain their required depth and by so doing a number of their characteristics are affected. The present study showed that the shallower the depth of planting the greater the movement made by the bulbs at the end of the season. Then it appears that too shallow planting of bulbs necessitates the bulbs responding to these shallow planting conditions by "pulling" themselves down into deeper ground. However, once plants are at an unfavourable depth in the soil (i.e. too deep) they seem to lack any mechanism for raising themselves in the soil profile. Perhaps in their natural environment weathering brings them closer to the surface, in a similar way as rocks and small stones rise to the surface. My data presented here suggests that planting bulbs too deep in the soil can have major implications on plant productivity.

In conclusion, the 5 cm depth is an ideal planting depth that could be recommended for the growth of either hyacinth or the lily bulb when they are planted in pots. Planting at this depth will give the best performance in terms of vegetative growth, flower quality and bulb yield at harvest. Alternatively, if only flower quality or the characteristics of the above ground portion of the bulb is the desired goal of the grower, hyacinth could also be planted at 0 cm depth (bulbs placed on soil surface) in pots. This is because in this study, even though the hyacinth bulb delayed in emergence when planted at 0 cm depth, leaf growth and flower quality were similar to those exhibited by the 5 cm. However, if the bulb yield is the aim of the grower, hyacinth bulb should not be planted at 0 cm depth since bulbs planted at this depth would not give any gain in fresh weight at harvest. Also, planting of lily bulbs at 0 cm should not be attempted by any grower because bulbs planted at this depth will not even sprout or survive and the grower will not achieve anything.

5.3.3 Nutrients feeding effects

The application of either ammonium sulphate [$(NH_4)_2SO_4$] or dibasic sodium hydrogen phosphate (Na_2HPO_4) especially at 60 or 90 mM increased vegetative growth (leaf growth and plant height) of hyacinth and the lily bulbs as compared to the control that did not receive any nutrients application, but in general, plants fed with $(NH_4)_2SO_4$ recorded higher vegetative growth, retained their florets for a longer time and had longer life cycle than the control and those fertilised with Na_2HPO_4. This observation is in support of Mahgoub *et al*. (2006) who worked on Irish bulb and reported that plant height, leaf fresh and dry weight, and inflorescence length increased when the bulb were fertilised with nitrogen at the rate of 40 g plus 30 g K/m^2. In the present study, the high vegetative growth of plants supplied with $(NH_4)_2SO_4$ also resulted in high bulb (mother bulb) weight at harvest as well as bulblets yield at harvest because the hyacinth and lily bulbs supplied with $(NH_4)_2SO_4$ recorded higher gain in bulb fresh weight as well as weights of offsets than those fed with Na_2HPO_4. The observed increases in these quantities as the level of applied $(NH_4)_2SO_4$ increased is due to the fact that $(NH_4)_2SO_4$ is a nutrient source for nitrogen and nitrogen is known to play a major role during growth and development of plants (Scott 2008). In fact, nitrogen is a constituent of other plant compounds such as chlorophyll, cell wall and nucleotides (Havlin *et al.* (1999). In the soil, the element is absorbed as NH_4^+ ions (Bennett, 1993) and these ions are then transformed in the plants to the amine form and utilised to form amino acids which are essential for protein formation (Bergmann, 1992). Since enzymes play a major role in a number of metabolic processes, it is not surprising that nitrogen played a key role in the growth and development of these plants. In general, it has already been established that after planting, the level of reserve carbohydrates stored in flowering bulbs decrease during and after sprouting (Chapter 3) because the developing plants depend on these reserves as nutrients source for their growth and development. There is the need to replace these lost nutrients even before photosynthesis starts to take place. Replenishing the nutrients lost from the bulb (scales) during sprouting and also the growth of the developing plant, through nutrients feeding (application) as was carried out in this work, will further enhance the growth and development of bulbs and this was exactly the case of the plants that were fed with nutrients in this work because plants fed with nutrients had better growth and development than their control counterparts that received no nutrients application. The observed increase in vegetative growth and bulb yield of plants fed with nitrogen was also due to their increased chlorophyll formation

that also reflected in their higher rates of photosynthesis as compared with the control and plants fed with Na_2HPO_4. Chlorophyll content of plants is an indicator of photosynthetic rate. As stated under section 5.3.2 and 5.3.3, chlorophyll absorbs energy from sunlight for the process of photosynthesis to occur. Thus in the lily, plants fed with $(NH_4)_2SO_4$ did not only record high levels of chlorophyll content and growth, but these plants were also characterised by high rates of photosynthesis and stomatal conductance.

Results of this study also showed that in hyacinth, the application of Na_2HPO_4 promoted inflorescence development better than $(NH_4)_2SO_4$, because plants fed with Na_2HPO_4 had higher values of inflorescence height, inflorescence length and number of florets (flower quality) than the control or those fed with $(NH_4)_2SO_4$. In the present work, Na_2HPO_4 was used as a source of phosphorus and this nutrient element is known to be a component of nucleic acids and genes. Also, it has been established that phosphorus plays a major role in reproduction of plants as it enhances the production and maturation of flowers and fruits, and also improves their quality (Bennett, 1993). Hamit (2001) also reported that the application of phosphorus increased the number of spikes but the number of florets per plant in *Freesia hybrida* was not affected. However, the present studies also showed that in the lily bulb, flower quality was not enhanced by the application of Na_2HPO_4 but rather it was the application of $(NH_4)_2SO_4$ that improved flower quality. This is because in the lily, floret length and the length of peduncle were higher from plants fed with $(NH_4)_2SO_4$ than the control and those that received application from Na_2HPO_4. The observed difference in the response to flower quality by these two species of bulbs in the present study, among other factors is due to variations in their genotypic constitution (Treder, 2000).

In conclusion, $(NH_4)_2SO_4$ at the rate of 60 and 90 mM but particularly the latter rate, gave the best performance in terms of vegetative growth and flower quality in the lily according to the results of this study. Also, the application of this nutrient at the same rate enhanced leaf growth, bulb yield and offsets production in hyacinth, however, it was the supply of Na_2HPO_4 at the rate of 60 or 90 mM that gave the best performance in terms of flower quality in hyacinth. Based on the results of this study, it would be recommended that the growth of hyacinth or lily in pots should be accompanied by the application of $(NH_4)_2SO_4$ at the rate equivalent to 60 or 90 mM (or any equivalent

nitrogen rate from any nutrient source). In the case of hyacinth, both $(NH_4)_2SO_4$ and Na_2HPO_4 should be applied to these plants to enhance flower quality as well as the growth of vegetative parameters. The study did not, however, investigate the combined effects of these nutrients on growth and yield of the bulbs and thus it would be difficult at this stage to make any recommendations on the rates that would be suitable for optimum growth of these bulbs when the two nutrients are applied at the same time. Future studies on effects of N and P in hyacinth and lily should have to determine these rates when they are both applied to the bulbs.

5.3.4 Varietal effects

Studies on the evaluation of eight varieties of hyacinth based on their characteristics in terms of bulb or flower production revealed that variety Sky Jacket produced the highest growth of leaves whilst Jon Bos and Pink Pearl produced relatively low vegetative growth. Purple Voice was the second best variety in terms of leaf growth. The other varieties, however, produced similar leaf growth. Variations in growth of the varieties may be due to differences in their genetic makeup (Troughton, 1970), and probably genotype x environment interaction. Also, the highest leaf growth of variety Sky Jacket means that it had a higher photosynthetic apparatus and leaf canopy architecture than the other varieties. Similarly, this variety recorded the highest chlorophyll concentration. Photosynthetic rate was not recorded of these varieties at the time of data collection (due to equipment failure), but the high photosynthetic apparatus (leaf biomass or surface) coupled with the high chlorophyll content of Sky Jacket might have enhanced its rate of photosynthesis and this was reflected not only in the weight gain of the mother bulb of this variety but also the number and weight of bulblets as recorded at the time of harvest. Based on the same explanation, Jon Bos and Pink Pearl recorded the least gain in weight at harvest because they had low chlorophyll content and low photosynthetic apparatus. This observation is in accordance with that made by Stahlschmidt *et al*. (1994). According to these authors, garlic variety Rosado had low bulb dry weight at harvest because it recorded low leaf area ratio that resulted from the cultivar's smaller photosynthetic apparatus. The present study, however, showed that varieties Fondant and Blue Jacket produced the highest flower quality (inflorescence height, inflorescence length and number of florets) but lower leaf growth and fresh weight gain as compared to Sky Jacket. The relatively low gain in bulb weight at the

time of harvesting in the cases of Fondant and Blue Jacket may not only be due to their low photosynthetic leaf surface or chlorophyll concentration, but also probably due to the fact that assimilates from current photosynthesis and/or reserves stored in the bulb prior to planting were diverted for inflorescence development instead of leaf growth, and leaf growth (photosynthetic apparatus), in general, is an indication of bulb yield. This is evident from the fact that inflorescence is an important sink organ in flowering bulbs (Die *et al*., 1970), and since inflorescence depends on the reserves stored in the bulb scales and/or current photosynthesis for their growth and development (Wassink, 1965), it follows also from this illustration that, if inflorescences have competitive advantage over the leaves in terms of acquisition of resources for growth, before photosynthesis sets in, as occurs when the leaves are too young to photosynthesize, then inflorescence will develop or grow at the expense of leaf growth. This scenario might have occurred in the cases of varieties Fondant and Blue Jacket.

In conclusion therefore, results about the characteristics of these hyacinth varieties showed that it will be advisable if growers consider Sky Jackets and Purple Voice in the selection of varieties to grow if their desired aim is to get high bulb yield at harvest (bulb weight gain) or if bulblets production is their ultimate aim in growing of bulbs. Otherwise, growing Fondant or Blue Jacket should be resorted to for optimum flower quality. Flowers of hyacinth are used in the perfumery industry. This means that, the greater the quantity of flower production of the variety, the higher the amount of oil that will be extracted. Thus, varieties Fondant and Blue Jacket must be considered by growers who wish to produce these flowers for perfumery uses. But for indoor decorations, varieties such as Pink Pearl and Jon Bos will be ideal because they are small (they have low plant height and leaf growth) and produce small flowers that could easily be handled especially when they are placed on table tops in the room.

CHAPTER 6

ISOLATION AND SEQUENCE ANALYSIS OF HYACINTH STARCH METABOLISM GENES

6.1 Introduction

Chapter 3, which investigated carbohydrate metabolism of hyacinth scales showed that starch content of the scales ranged from 306.6 µmol g^{-1} fresh weight (outermost scale) to 214.2 µmol g^{-1} fresh weight (innermost scale); whilst glucose, fructose and sucrose levels of the scales ranged from 14.3-11.8 µmol g^{-1} fresh, 4.9-0.7 µmol g^{-1} fresh and 17.4-12.5 µmol g^{-1} fresh weight, respectively, prior to planting of the bulbs. Also, at the time of above-ground senescence, the sugars almost disappeared completely, whilst starch accumulated in the scales. This implies that starch is the major storage carbohydrate in the common hyacinth. A study was initiated to investigate the biochemical mechanisms underlining the synthesis and breakdown of starch in this flowering bulb. Starch is the end product of photosynthesis, which occurs primarily in the source tissues of plants, but it is stored in the sink tissues. The polysaccharide is not only a source of dietary carbohydrates, but it has many industrial uses. As for example, starch is used for the manufacture of packaging materials and for making ethanol. Martin and Smith (1995) stated that, there is a relationship between the basic characteristics of starch and its architectural organisation. Therefore, the organised arrangements of amylopectin and amylose into higher order molecular structures gives rise to granule formation, and this makes the molecule resistant to degradation.

Plants are very sensitive and responsive to their surroundings because they are immobile. The inability of plants to move from one place to another place implies that they have few strategies for survival other than acclimatisation. In recent years, the interest in the genes that are involved in starch biosynthesis and degradation has increased. This is because the genes involved in carbohydrate regulation and metabolism in plants provide important mechanisms for plants to adjust to various environmental changes (Koch, 1996). The genes that encode ADPG-pyrophosphorylase (AGPase), the most important enzyme involved in starch biosynthesis, are sugar responsive (Krapp *et al*, 1993), and the expression of this enzyme is enhanced by sugars in transgenic potato cell cultures and in other species (Müller-Röber *et al*., 1990). Starch synthase and starch branching enzyme are also induced and/or expressed at elevated levels when sugars are plentiful (Salehuzzaman, 1994). Also, according to Beck and Zieglstarch (1989), starch degradation occurs as a result of the joint action of phosphorolysis and hydrolysis. The biochemistry of carbohydrate metabolism, particularly in relationship to the synthesis and/ or breakdown of starch in most plants,

is poorly understood (Miller, 1992). Ohdan *et al*., (2005) stated that the metabolic pathways involved in biosynthesis and degradation of starch are different between source and sink tissues, and the mode of gene expression of enzymes that are involved in carbohydrate metabolism was dependent on the type of tissues concerned as well as the developmental stage of the plant. In addition, it has been established that, in plants, enzymes involved in starch metabolism are grouped into two: enzymes of starch synthesis, and those responsible for degradation of starch. Enzymes of starch synthesis include ADP-glucose pyrophosphorylase (AGPase), the enzyme that is involved in the formation of ADP-glucose and pyrophosphate from ATP and glucose-1-phosphate; starch synthase, the enzyme involved in the linking of ADP-glucose to the non-reducing end of the growing starch chain by α-(1, 4) bond and which act on both amylose and amylopectin; starch branching enzyme (SBE), the enzyme responsible for the production of the (1, 6) branch points in amylopectin molecules (Myers *et al*., 2000; Nakamura, 2002; Smith, 1990). The degrading enzymes include starch phosphorylase, the enzyme that catalyses a reversible reaction producing glucose-1-phosphate from the non-reducing ends of the starch molecules (Duffus, 1984; Steup, 1990); and the amylases, the enzymes that catalyse the hydrolysis of α-(1, 4)-linked glucans directly, thereby yielding sugars and oligosaccharides (Davies, 1990). Sowokinos (2007) also observed that the characteristics of enzymes involved in the metabolism of carbohydrates of plants, influence the process of regulation and partitioning of carbohydrates in such plants. But in hyacinth, however, information on the specific details on isolation and characterisation of carbohydrates metabolising enzymes is not available and this needs to be addressed. Having a good knowledge about the mechanisms that govern the synthesis and/ or degradation of starch in hyacinth is important, because it will provide understanding about the basic principles of the physiology of hyacinth in particular, and flower bulbs in general.

Being able to study the level of transcription of a particular gene has become by the use of techniques such as RT-PCR and advanced techniques such as realtime PCR can be used to quantify the level and control of gene expression in individual structures in the plant under different conditions. For these techniques to be used, specific probes need to be generated and for this a degree of sequence information is needed. A molecular level studies were therefore initiated to generate sequence information from enzymes that are involved in the synthesis and degradation of starch in hyacinth.

6.2 Materials and Methods

6.2.1. Chemicals

Chemicals used in this study included 10 x PCR buffer, dNTPs, $MgCl_2$, and Taq polymerase. These were ordered from Fermentas. Also TOPO TA vector, sterile water, salt solution and a vial of competent Top 10 cells from Invitrogen were used. Other chemicals used were Buffer AP 1, Buffer AP 2, Buffer AP 3, Buffer AW, Buffer AE, Buffer QG, Buffer PE, Buffer EB, Buffer PB and RNase A stock solution from the Qiagen GmbH, D-40724, Hilden. Ethidium bromide, TBE, Agarose, SOC medium, ampicillin, Xgal, dimethyl formide, IPTG, glucose, Tris HCl, EDTA, NaOH, SDS, and KOAc were also ordered from Sigma-Aldrich.

6.2.2. Plant materials

The plant materials used were the leaves of the common hyacinth (*Hyacinthus orientalis*), from which DNA was extracted for the study.

6.2.3 Extraction of hyacinth DNA

To isolate genes fragments from enzymes involved in starch metabolism of hyacinth, DNA from hyacinth was first extracted. The Qiagen DNEasy plant mini kit was used for the extraction. Approximately 0.1 g of the leaves of hyacinth was placed in a 1.5 ml microfuge tube and kept in a bucket containing dry ice. The leaves were ground using a sterile plastic pestle until no lumps remained and 400 µl of Buffer AP 1 was added. An amount of 4 µl of RNase A stock solution (100 mg/ml) was added. The tube was incubated for 10 min at 65 ^{o}C and 130 µl of Buffer AP 2 added and incubated on wet ice for 5 min. This allowed detergents, proteins and polysaccharides to be precipitated. The mixture was centrifuged at at 13,000 rpm for 5 min. The supernatant in a QIA shredder column was centrifuged for 2 min at 13,000 rpm. The flow through was transferred to a new 1.5 ml tube and volume determined with a pipette. Then, 1.5 volumes of Buffer AP 3 was added and mixed followed by the addition of 650 µl of the lysate, applied in a DNeasy mini spin column and centrifuged for 1 min at 8000 rpm. The flow through was discarded but the collection tube was kept. The DNeasy column was placed in a new 2

ml collection tube and 500 µl of Buffer AW added to the column and centrifuged again at 8000 rpm for 1 min. The flow through was discarded, the collection tube was kept and another 500 ul of Buffer AW added to the column and centrifuged again for 2 min at 13,000 rpm to dry the membrane. The column was transferred to new 1.5 ml tube and 40 µl of Buffer AE added onto the membrane. This was incubated for 5 min at room temperature followed by centrifugation at 8000 rpm for 2 min to elute the DNA (See Fig 6.1).

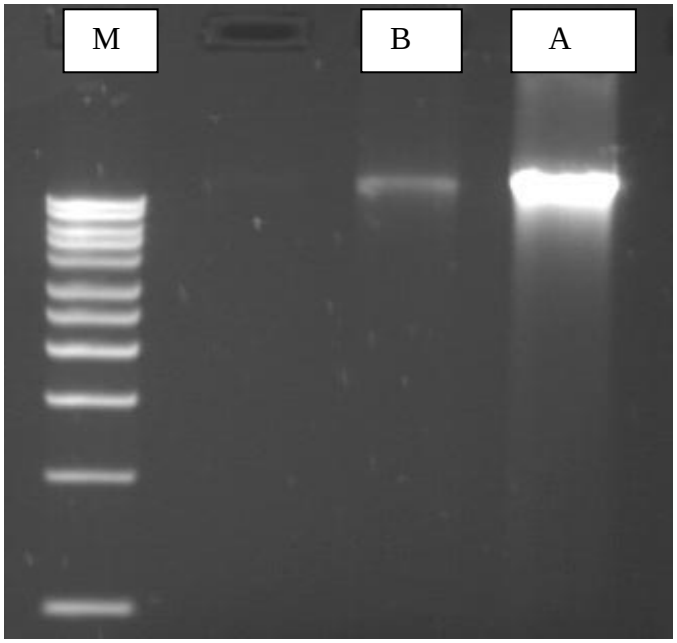


Fig 6.1: Hyacinth DNA of volume 5µl (A) and 0.5µl (B) resolved by using 1% TBE agarose gel electrophoresis. M represents the marker.

6.2.4 Agarose Gel Electrophoresis

Genomic DNA and PCR products were resolved using 1% TBE agarose gel electrophoresis. Ethidium bromide was incorporated into the gel matrix (0.4mg/ml (w/v) and gels were run at 100V and visualised on a transilluminator (λ = 365nm).

6.2.5.1 Identification of conserved domains

Multiple DNA alignments were carried out using the Entrez website of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/sites/gquery), the

protein sequence database for the enzymes in question were searched for. Then, conserved domains for proteins of a number of plants, ranging from monocotyledonous species to the dicotyledonous plants, were identified using the Multiple Alignment Viewer.

6.2.5.2 PCR primer design

Degenerate primers were designed to conserved domains of enzymes using 'Translator' (http://www.justbio.com/translator/index.php). The Tm of primer pairs were matched by adjusting the length of the primer.

6.2.6 Polymerase chain reaction (PCR)

PCR uses a thermostable Taq polymerse to ampify DNA sequences by thermo-cycling. DNA is kept in a test tube, sealed, and the tube placed in a thermal cycler. The cycler is programmed to cycle between three different temperatures, first a high temperature (about 95 ^{o}C) to separate the DNA strands; then a relatively low temperature (about 40 ^{o}C) to allow the primers to anneal to the temperate DNA strands; and then to a medium temperature (about 72 ^{o}C) to allow the DNA synthesis (Weaver, 1999).

6.2.7 Isolation of DNA fragments from Agarose Gel slices

DNA fragments resolved by agarose gel electrophoresis were viewed using a transilluminator and cut out with a scapel from the gel (λ = 365nm). The DNA was recovered using a Qiagen Gel purification Kit. The gel slice containing the PCR product was weighed and 3x volume of buffer QG added and incubated at 50 ^{o}C for 10 min. The mixture was applied to column and spun at 1 min, discarding the flow through. An amount of 0.5 ml of buffer QG was added and spun at 1 min, discarding the flow through. An amount of 750 µl of buffer PE was then added and spun at 1 min and the flow through discarded. Spinning was again done at 13,000 rpm for 1 min and the samples placed into a new tube. The DNA was eluted by adding 50 µl of buffer EB, left for 1 min, followed by spinning for another 1 min.

6.2.8.1 Subcloning of the PCR fragments using the TOPO TA vector

TOPO TA vector system (Invitrogen) was used to subclone the PCR fragments. The TOPO TA cloning reaction was made by adding 2 µl of the PCR product to 1 µl salt solution, 1.5 µl of sterile water and 0.5 µl of TOPO vector. The reaction mixture was gently mixed and incubated for 5 min at room temperature to allow the inserts to ligate into the plasmid.

6.2.8.2 Plasmid Transformation

A vial of competent Top 10 (Invitrogen) cells were quickly thawed to 4 ^{o}C and kept on ice. The contents of the cloning reaction were added to the competent cells, mixed gently by flicking the tube and incubated on ice for 15 min. The cells were heat shocked in a 42 ^{o}C waterbath for 30 seconds and immediately transferred to ice and 250 µl of room temperature SOC medium was added; tubes were capped and shaken horizontally but gently at 37 ^{o}C for 1 hour. Colonies containing recombinant plasmids were selected/identified by plating 50µl of transformed cells onto LB Amp X Gal plates and incubating at 37ºC for at least 8 hours. Then, 500 ml LB agar was melted in the microwave and allowed to cool to approximately 55 ^{o}C and 500 µl ampicillin (50 mg/ml) was added, gently shaken to mix, and 25 ml poured into each petri dish in laminar flow hood, and were allowed to set and dry. Then Xgal solution was made by combining 0.2 g Xgal in 9 ml dimethyl formide and 0.02 g IPTG in 1 ml water. An amount of 100 µl of the Xgal solution was then spread onto each of the 5 LB amp plates and was allowed to dry in the laminar flow hood. Colonies containing plasmids with cloned inserts were identified by white colour. These were purified by streaking on LB amp plates.

6.2.9 E. *coli* plasmid miniprep DNA preparation

The alkaline method was used for this preparation. An amount of 100 µl of ampicillin (50 mg/ml) was added to 100 ml LB broth; then 2 ml of the media was pipetted into sterile tubes. Tiny amounts of the bacterial cells (colonies identified from above) were transferred into the tubes using a wire. The bacterial cells were allowed to grow for a period of 48 hours at 37 ^{o}C with shaking. Then 1 ml of the culture was taken into a

clean 1.5 ml eppendorf tube, spun at maximum speed for 1 min, and the supernatant discarded. Then 100 µl of solution I (a mixture of 50 mM glucose, 25 mM Tris HCl @ pH of 8 and 10 mM EDTA) and 200 µl of solution II (a mixture of 0.2 M NaOH and 1 % SDS) were added. The tube content was thoroughly mixed and incubated for 5 min. An amount of 150 µl of solution III (a mixture of 5M KOAc @ pH of 4.8) was added and incubated on ice for 5 min followed by centrifugation in a microfuge at 13000 rpm for 5 min. The supernatant was poured into new tubes leaving all white precipitate behind. Then 100 µl of phenol (lower phase) was added, mixed by inversion, centrifuged for 5 min, and the upper phase taken into fresh tubes. 50 µl of chloroform was added, mixed by inversion, centrifuged for 5 min at 13000 rpm, and the upper phase taken into fresh tubes, 1 ml of 100 % ethanol was added, mixed and centrifuged for 10 min. The supernatant was discarded and 100 µl of 70 % ethanol added, followed by centrifugation for 2 min at 13000 rpm. The supernatant was again discarded. Pellets were dried at 37 ^{o}C in a heating block for 30 min with lid open but covered loosely with a piece of aluminium foil to prevent contamination by any foreign particles. Then, distilled water (45 µl) was added, resuspended gently by flicking the tube and 0.2 µl of RNase A added followed by incubation at 37 ^{o}C for 30 min. Then, 5 µl of 3M NaOH and 3x volume of 100 % ethanol were added and spun for 10 min. The supernatant was discarded followed by the addition of 500 ml of 70 % ethanol, spun for 2 min and supernatant discarded. Pellets were dried at 37 ^{o}C and 20 µl of distilled water added and kept for sequencing.

6.2.10 Purification of the PCR product

Buffer PB (5 vol) were added to one volume of the PCR samples and placed in a spin column in 2 ml collection tube and spun for 1 min at 13000 rpm, and the flow through was discarded. Then, the sample was washed using 0.75 ml of buffer PE, spun for 1 min at 13000 rpm, and the flow through was discarded. The mixture was spun for another 1 min before placing in a new tube. Buffer EB (50 µl) was added on the membrane and spun for 1 min. The sample was then collected and kept for sequencing.

6.3 Results

6.3.1 Design of PCR primers and PCR reactions in year 2008

In July 2008, primers were designed for the following enzymes: ADP glucose pyrophosphorylase, starch phosphorylase, starch branching enzyme and starch synthase for their use in various PCR reactions, with a view to isolating the gene fragments of these enzymes. Conserved domains of these enzymes were obtained. The domains used for the design of the primers were highly conserved across a number of plants, ranging from monocotyledonous species to dicotyledonous plants. These domains (highlighted in black colours and in capital letters, Tables 6.1-6.4) are presented, and the base nucleotides that code for the various amino acids in the domains are also shown.

6.3.1.1 PCR primers design for ADP glucose pyrophosphorylase

The forward primer for the ADP glucose pyrophosphorylase enzyme, ADPGPY 100F was designed using the domains YPTKKAKP, located at position 100 of the total alignment of the proteins conserved for this enzyme (Table 6.1a). Comparing the base nucleotides that code for the specific amino acids in the conserved domains, and choosing the ones common among them, the forward primer for the ADP glucose pyrophosphorylase enzyme, ADPGPY 100F was obtained as ADPGPY 100F = 5'TAYCCNACNAARAARGCNAARCC (Tm = 52.8^{o}C, Mol wt = 6999 µg), where Y = C,T; N = C, T, A, G; R= A, G. The reverse primer, ADPGPY 363R was designed using the domain AFYNANGI, which occurs at position 363 of the total alignment of the proteins conserved for the enzyme (Table 6.1b). The forward primer ADPGPY 363F was first designed before getting the reverse primer from it. Thus, comparing the nucleotides (Table 6.1b) and selecting the ones common for each amino acid, ADPGPY 363F was obtained as 5'GCNTTYTAYAAYGCNAAYGGNAT, where N, Y have the same meanings as stated above. The reverse primer ADPGPY 363R was then obtained by reversing the nucleotides in the forward primer. Thus ADPGPY 363R was designed as ADPGPY 363R = 5'ATNCCYTTNGCYTTYTAYAANGC (Tm = 57^{o}C, Mol wt = 7117 µg).

Table 6.1: Conserved protein domains of ADP glucose pyrophosphorylase enzyme at (a) position 100, and (b) position 363 of the whole protein alignment.

(a) Domains at position 100 of the proteins conserved for ADP glucose pyrophosphorylase

Position 100	**Y**	**P**	**T**	**K**	**K**	**A**	**K**	**P**
	TAT	CCT	ACT	AAA	AAA	GCT	AAA	CCT
	TAC	CCC	ACC	AAG	AAG	GCC	AAG	CCC
		CCA	ACA			GCA		CCA
		CCG	ACG			GCG		CCG

(b) Domains at position 363 of the proteins conserved for ADP glucose pyrophosphorylase

Position 363	**A**	**F**	**Y**	**N**	**A**	**N**	**G**	**I***
	GCT	TTT	TAT	AAT	GCT	AAT	GGT	ATT
	GCC	TTC	TAC	AAC	GCC	AAC	GGC	ATC
	GCA				GCA		GGA	ATA
	GCG				GCG		GGG	

*The nucleotides highlighted in red colour in this table were not considered in the primer design.

6.3.1.2 PCR primers design for starch phosphorylase

The primers for the starch phosphorylase enzyme were designed using the conserved domains located at positions 255 (forward primer) and 680 (reverse primer) of the total alignment of the domains conserved for this enzyme (Table 6.2a and b). The forward primer STPH 255F was obtained in the same way as stated above for ADP glucose pyrophosphorylase. Thus, from Table 6.2a, the forward primer was designed from the domain (F/Y) QGANAG, found at position 255 of the total alignment of the protein conserved for starch phosphorylase. Thus, the forward primer STPH 255F = 5'TWBCARGGIGCIAAYGCIGG (Tm = 63.5^{o}C, Mol wt = 6208 µg), where W = T, T, A, A; B = T, C, T, C; R = A, G; I = T, C, A, G; Y = T, C. The reverse primer, STPH 680R was obtained by reversing the nucleotides in the forward primer STPH 680F, which was obtained from the domains QNKTNGP (Table 6.2b) by comparing the nucleotides of the various amino acids of the domains. Thus primer STPH 680F =

5'CARAAYAARACIAAYGGICC, where R, Y and I have the same definitions as stated above. The reverse of the forward primer STPH 680F gave the reverse primer STPH 680R, that is, STPH 680R = 5'GGICCYTTIGTRTTYTTRTG (Tm = 55.3^{o}C, Mol wt = 6129 µg).

Table 6.2: Design of primers for the starch phosphorylase enzyme using the conserved domains at (a) position 255, and (b) position 680 of the total protein alignment of the domains conserved for starch phosphorylase.

(a) Domains conserved at position 255 for the starch phosphorylase enzyme

Position 255	**(F/Y)**	**Q**	**G**	**A**	**N**	**A**	**G***
	TTT	CAA	GGT	GCT	AAT	GCT	GGT
	TTC	CAG	GGC	GCC	AAC	GCC	GGC
	TAT		GGA	GCA		GCA	GGA
	TAC		GGG	GCG		GCG	GGG

*The nucleotides highlighted in red colour in this table were not used in the primer designed.

(b) Domains conserved at position 680 for the starch phosphorylase enzyme

Position 680	**Q**	**N**	**K**	**T**	**N**	**G**	**P***
	CAA	AAT	AAA	ACT	AAT	GGT	CCT
	CAG	AAC	AAG	ACC	AAC	GGC	CCC
				ACA		GGA	CCA
				ACG		GGG	CCG

*The nucleotides highlighted in red colour in this table were not used in the primer designed.

6.3.1.3 PCR primers design for starch branching enzyme

In the design of primers for starch branching enzyme, use was made of the domains found at positions 135 (forward) and 453 (reverse) as shown in Tables 6.3a and b, respectively. The forward primer SBE 135F according to Table 6.3a, and using the domain (D/G) (H/D) PIYDDP at position 135 of the total alignment of the conserved domain, primer SBE 135F was obtained. Thus, the forward primer, that is SBE 135F =

5'GRNSAYCCNATHTAYGAYGAYCC (Tm = 54.9^{o}C, Mol wt = 6994 µg), where R = A, A, G, G, G, G; N = T, C, T, C, A, G; S = C, C, G, G; Y = T, C, T, C; N = T, C, A, G; H = T, C, A. The reverse primer, SBE 435R was obtained in the same manner as those of ADP glucose pyrophosphorylase and starch phosphorylase enzymes. Therefore according to Table 6.3b and using the domain GGYHKW at position 435, primer SBE 435F = 5'GGNGGNTAYCAYAARTGG. To get the reverse primer SBE 435R, the nucleotides for the forward primer was reversed. Thus primer SBE 435R was obtained as follows: SBE 435R = 5'CCARTTYTGYTANCCNCC (Tm = 54.2^{o}C, Mol wt = 5413 µg).

Table 6.3: Design of primers for the starch branching enzyme using the conserved domains at, (a) position 135, and (b) position 435 of the total protein alignment of the domains conserved for starch branching enzyme.

(a) Domains conserved at position 135 for the starch branching enzyme

Position135	**(D/G)**	**(H/D)**	**P**	**I**	**Y**	**D**	**D**	**P***
	GAT	CAT	CCT	ATT	TAT	GAT	GAT	CCT
	GAC	CAC	CCC	ATC	TAC	GAC	GAC	CCC
	GGT	GAT	CCA	ATA				CCA
	GGC	GAC	CCG					CCG
	GGA							
	GGG							

*The nucleotides highlighted in red colour were not used in the primer designed.

(b) Domains conserved at position 435 for the starch branching enzyme

Position 435	**G**	**G**	**Y**	**H**	**K**	**W**
	GGT	GGT	TAT	CAT	AAA	TGG
	GGC	GGC	TAC	CAC	AAG	
	GGA	GGA				
	GGG	GGG				

6.3.1.4 PCR primers design for starch synthase

The forward primer SS 98F was designed using the domain GGDVG (A/S) P (Table 6.4a). Thus primer SS 98F = 5'GGIGGIGAYGTIGGIKCICC (Tm = 64.3 ^{o}C, Mol wt = 6241 µg), where I = T, C, A, G; Y = T, C; K = G, T. As described for the other

enzymes, the reverse primer, for the starch synthase enzyme, primer SS 470R, was designed using the domain GWVGFSVHT that occur at position 470 of the multiple alignment of the domains conserved for starch synthase (Table 6.4b). But first, the forward position 470, i.e. primer SS 470F was obtained as shown below: i.e. SS 470F = 5'GGITGGGTIGGITTYTCIGTICAYAC, where, I and Y have their usual meanings as as mentioned above. The reverse primer, that is primer SS 470R was thus obtained as SS 470R= 5'GTYTGIACIGAYAAICCIACCCAICC (Tm = 64.1^{o}C, Mol wt = 8015 µg).

Table 6.4: Conserved protein domains of the starch synthase enzyme at (a) position 98, and (b) position 470 of the whole protein alignment.

(a) Domains conserved at position 98 for the starch synthase enzyme

Position 98	**G**	**G**	**D**	**V**	**G**	**(A/S)**	**P***
	GGT	GGT	GAT	GTT	GGT	GCT	CCT
	GGC	GGC	GAC	GTC	GGC	GCC	CCC
	GGA	GGA		GTA	GGA	GCA	CCA
	GGG	GGG		GTG	GGG	GCG	CCG
						TCT	
						TCC	
						TCA	
						TCG	

*The nucleotides highlighted in red colour were not used in the primer designed.

(b) Domains conserved at position 470 for the starch synthase enzyme

Position 470	**G**	**W**	**V**	**G**	**F**	**S**	**V**	**H**	**T***
	GGT	TGG	GTT	GGT	TTT	TCT	GTT	CAT	ACT
	GGC		GTC	GGC	TTC	TCC	GTC	CAC	ACC
	GGA		GTA	GGA		TCA	GTA		ACA
	GGG		GTG	GGG		TCG	GTG		ACG

*The nucleotides highlighted in red colour were not used in the primer designed

6.3.1.5 PCR reactions

In all, four PCR reactions were carried out in year 2008. The first PCR reaction (reaction 1) involved the use of the primers designed for ADP glucose pyrophosphorylase (ADPGPY 100F and ADPGPY 363R), the second PCR reaction

(reaction 2) made use of the primers designed for starch phosphorylase (STPH 255F and STPH 680R), the third reaction (reaction 3) also made use of the primers designed for starch branching enzyme (SBE 135F and SBE 435R), whilst in reaction 4, the primers designed for the starch synthase enzyme (SS 198F and SS 470R) were used. In these reactions, the expected PCR products, or band size required were 263bp, 425bp, 300bp and 272bp, respectively, for reactions 1, 2, 3 and 4. In each PCR reaction, the composition of the reaction mixture was as follows: 18.5 µl of distilled water, 5 µl of 10 x PCR buffer, 7 µl of dNTPs, 6.5 µl of $MgCl_2$, 1 µl each of the forward and reverse primers, 0.25 µl of taq polymerase and 1.25 µl of the hyacinth DNA, in a total volume of 40 µl per reaction. The PCR cycling conditions for each reaction were 94 ^oC for 4 min, 94 ^oC for 60 sec, 50 ^oC for another 60 sec, 72 ^oC for another 60 sec, then 72 ^oC for 7 min and 4 ^oC to hold the reaction. In all, the thermal cycler was programmed for 35 cycles. Reaction 1 was maintained at an annealing temperature of 40 ^oC, reaction 2 at 48 ^oC, whilst reactions 3 and 4 were kept at annealing temperatures of 44 ^oC and 62 ^oC, respectively. With the exception of reaction 4, all the reactions did not give any PCR product at a higher annealing temperature (> 55 ^oC), and too low annealing temperature gave rise to mixed priming, and so, the best annealing temperatures for the reactions were those stated above. The DNA fragments were isolated from the bands containing the PCR product (Fig 6.2) as detailed above, and TOPO TA vector system (Invitrogen) used to subclone the PCR fragments as also mentioned above.

6.3.1.6 Colony purification of the bacteria strain

PCR reactions were carried on the white colonies produced from reactions 1, 2, 3 and 4, using the same reaction conditions, and the original primers as the previous ones, however, instead of using the hyacinth DNA, tiny amounts of each colony was used as template and the number of cycles reduced from 35 to 15. The use of the colonies produced from their respective reactions as templates in these PCR reactions was to confirm sizes of bands produced as occurred during the previous PCR, and colonies that produced the right band size during these PCR reactions were identified (Fig 6.3). DNA was extracted from the identified colonies using the *E. coli* plasmid miniprep DNA preparation procedure described above. The DNA resolved by using 1% TBE agarose gel electrophoresis (Fig 6.4) and the results of sequencing (Fig 6.5) are presented.

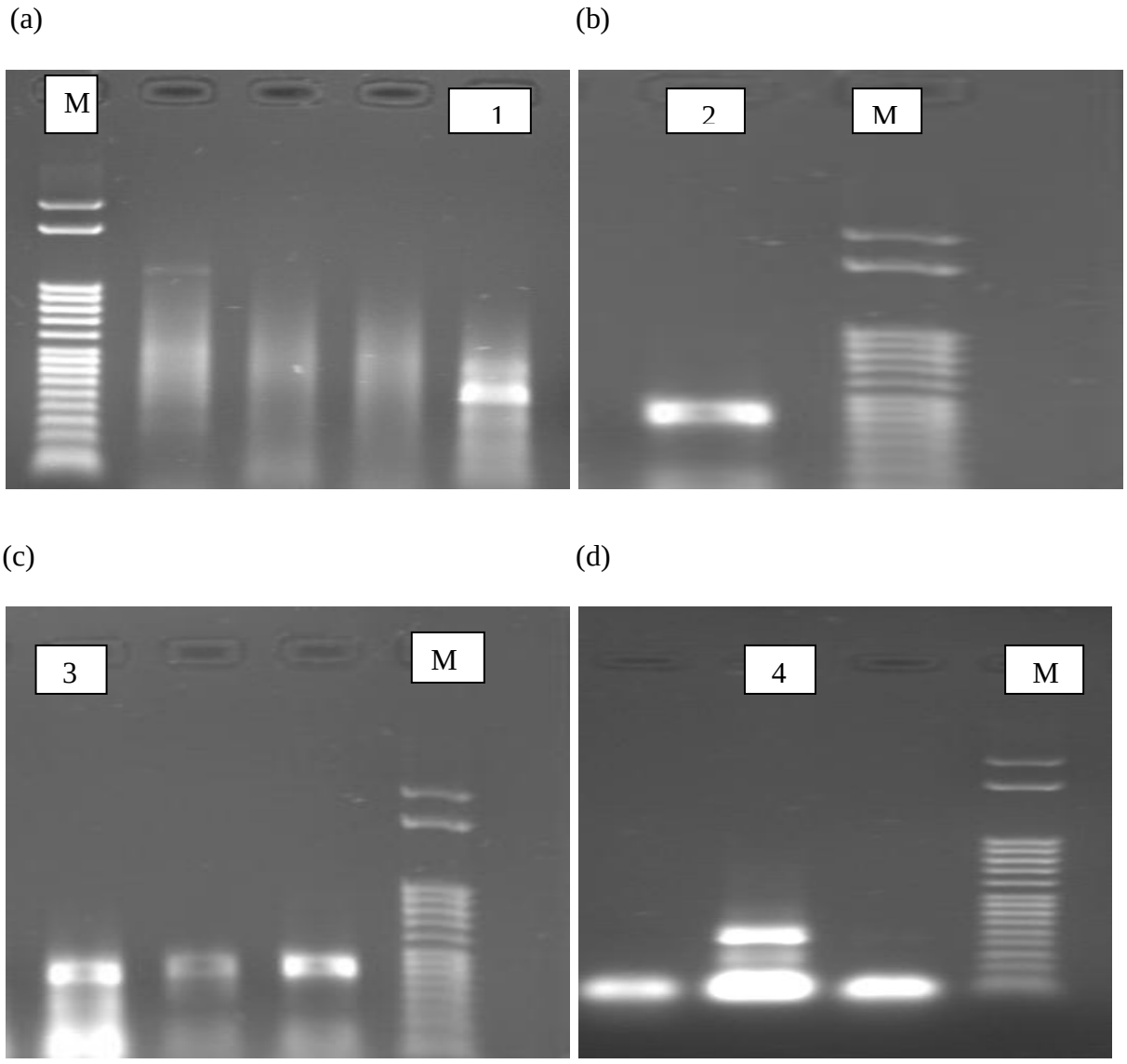
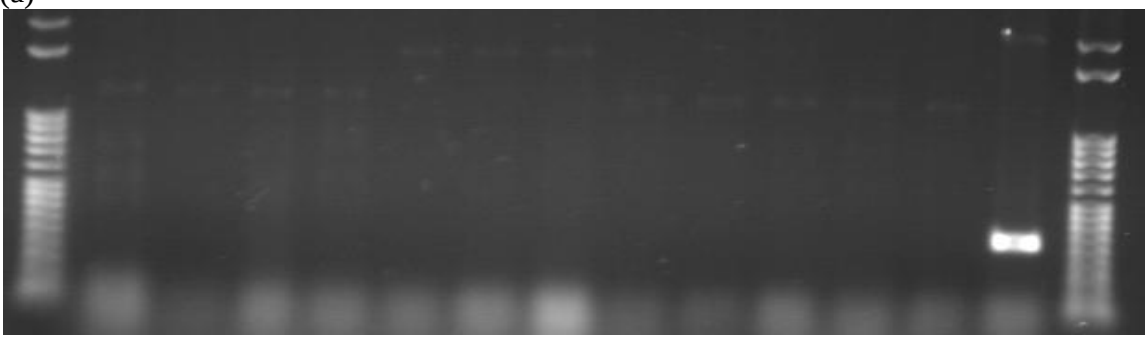
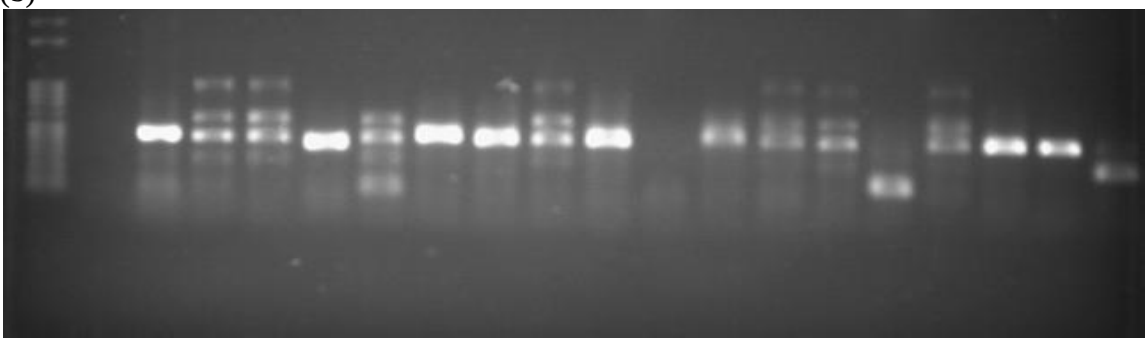


Fig 6.2: PCR products of reactions (a) 1, (b) 2, (c) 3 and (d) 4 using the primers designed for ADP glucose pyrophosphorylase, starch phosphorylase, starch branching enzyme and starch synthase, with expected band sizes of 263bp, 425bp, 300bp and 272bp, respectively. Photographs were taken of these bands prior to their cutting and gel purification. The bands were cut using a transilluminator (λ = 365nm) and the DNA fragments isolated from the gel slice prior to cloning. In each case, M represents the 50bp marker used.

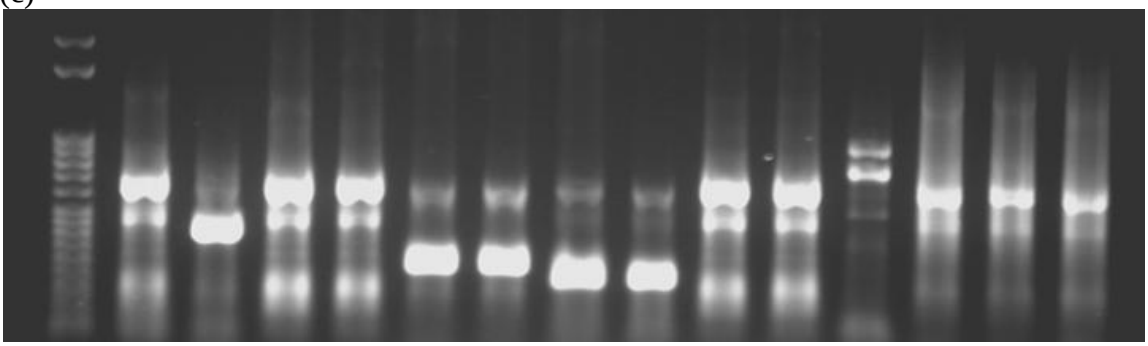
(a)



(b)



(c)



(d)

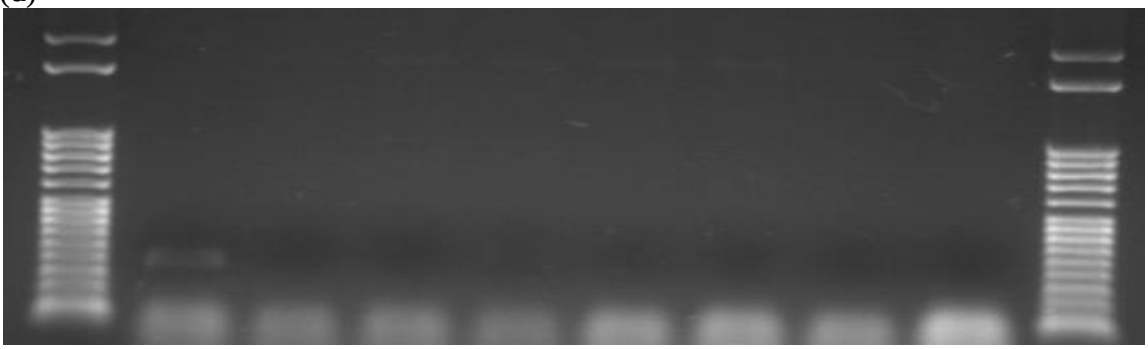
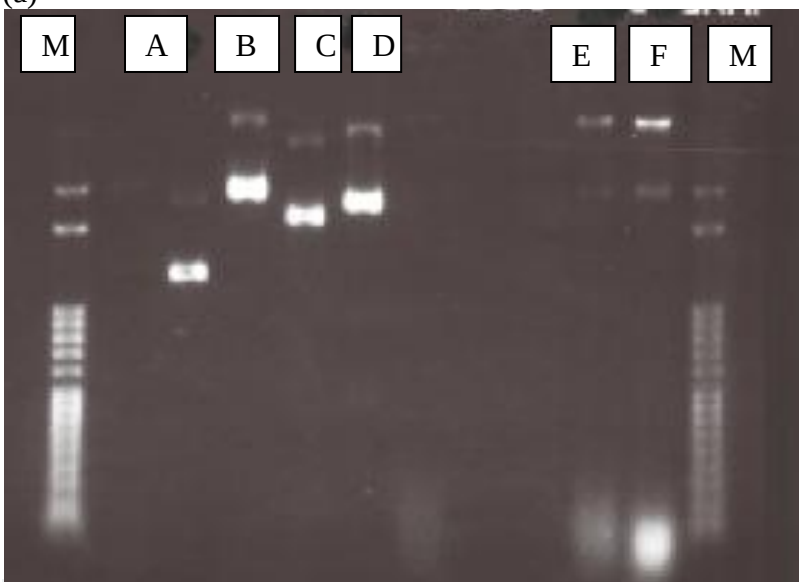


Fig 6.3: PCR of clones from reaction (a) 1, (b) 2, (c) 3, (d) 4 resolved by using 1% TBE agarose gel electrophoresis. DNA was extracted from the clones with the right band size for sequencing.

(a)



(b)

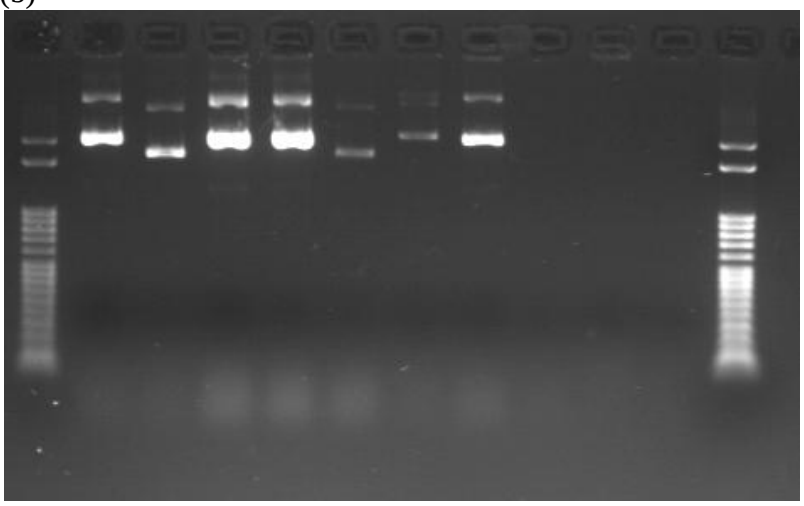


Fig 6.4: DNA extracted from the bacteria using the DNA mini prep procedure prior to sequencing was resolved using 1% TBE agarose gel electrophoresis, (a) DNA extracted from colonies from reaction 1 i.e. ADP glucose pyrophosphorylase (E and F), and those from reaction 3 i.e starch branching enzyme (B, C and D), and reaction 4 (A) i.e. starch synthase, after extraction from the bacteria. M is the marker and in (b) DNA extracted of colonies from reaction 2 i.e. starch phosphorylase.

(a)
TTGACATCGGGGCCGGAGGTGGTCAGTGCCGGCGGCGGCGAGGTCACGAAA
TCAGGTCGTTGCGGTCTGAATTGGAGAAGAAGATGGACCTCGATTCGGTGG
TTAGAGGTTGTGCTAGGGCAAATTAACATGGGGGTTTGGAAGATGGGATCA
AGACGAAGCTGTGGAGTATTGGATTGCGGGGTGAGATGATCGGGATCGCCG
GAATTTTGAGGAAGAAGTTAACGTAGATGATGGATGTGAATTTGGGGAAAT
CTGATCGGAAGGGTTTTGCCTTTTTAGTCGGGTAAAGGGCGAATTCTGCAGA
TATCCATCACACTGGCGGCCGCTCGAGCATGNATCTAGAGGGCCCAATTCG
CCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTG
ACTGG

(b)
TGAGGGCTTCGTGAACATGAGGGTTCCGGTAGGGTTTCTAGGGTTAGCTTGA
ACTCAAATTGAGCTCGGATCGGGATGTAGGGTTCAAGATAGCTCCAATTGG
GTTGGGGCTATGCTAGAGGGGTGGTTGACGATGCAGAGGGGATCGGAATCA
AGAGATGCCGTGTCAGGTGAGGGGACAGCGGGAGGTTGAAGATGATGAAG
CTTAGGGTTCCAAATTGGGATGTCGGGAGCTAGGGTAGCTTAGGGGAAATT
ATCGATGGGGTTAGAATCAGTGAGTGCTATGGAGTGATTTGACATCAGAAT
TAAACAAACGGCCCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGT
GGATCCGAGCTCGGTACCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCT
GTGTG

(c)
CCTGTTGAGCTCCACCTCCAACAGAAGAAGTGACCACAATCGGAGGAACCT
CTTGGAGAAGGGTTATCTCTAACAAAGGGAAAACATTATTAAAGGAACCTA
AACTTAGACCACGATCATTGGATATAGGTCACTAACCTGAAGAACCCGGTG
CTTGGAAAACCACAGAAAGAGGGTCCGCAACAACAACTTTCGACGCCGGAG
TAGGGGCAGCCTTTGAAGTCTCAACCGTCCCTGAGGGAGGAGTAGACACAC
TCACCACAGAAGCAGATGAGACCTCCGCAGAACGGGTCCTAGGCTTTTTAT
TCTTCTCCACCCTCTCTGATGAAGGAGAAGAATGCTTCCTCTTACCAGAAGA
TACAGAAGGAGAGGGAGGCCCCGGATTAGCGGGAATTACCGGGAGGAGGA
TCGTCGTAAATGGGATCCCCAAGGGCGAATTCTGCAGATATCCATCACACT
GGCGGCCGCTCGAGCATGCATCTAGAGGGCCCAATTCGCCCTATAGTGAGT
CGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCC
TGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGG
CGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAG

(d)
TCGAGTCCAATTAGCTGATGGGTGTGTTAGCTTGGAGGTGGAGGTCGTGTA
GAAGTGGCTAGGGGCAAGTTTTGGTTTACAGCTGGAGGTTGAAGGCGATGA
ACAGTGCTAGTTCATTCCGATGGGTTCTTGAGATCGCTGCTGACGGTAGAGG
GCTCCGTTGGGCATGCGGTTGGGTTCGTTGAGTAGCTGGCAGTGGGGGCGC
CCCCACATCCCCCAAAGGGGGG

Fig 6.5: Sequencing results of the clones identified from reactions (a) 1, (b) 2, (c) 3, and (d) 4, that is for ADP glucose pyrophosphorylase, starch phosphorylase, starch branching enzyme and starch synthase, respectively. The above sequences do not have any similarity to the enzymes in question, so they probably might have been generated by the primers hybridising to other genomic sequences.

6.3.2 Design of PCR primers and PCR reactions in year 2009

The isolation of the gene fragments of the various enzymes was not successful in year 2008. This is because the BLAST search for the sequences produced during year 2008 (Fig 6.5) did not give information about the targeted enzymes. Thus, there was the need to redesign different primers for these enzymes for a fresh start of this part of the study in year 2009 and the early part of year 2010.

6.3.2.1 PCR primers design

In November 2009, primers were redesigned for two of the enzymes studied in year 2008, starch phosphorylase and starch synthase. Conserved domains of these enzymes were searched for, as already described. The domains that were highly conserved across a number of plants or common to all the plants considered were recorded along side their positions at which they were located on the total alignment length of the whole protein. Using these domains, the DNA sequences of the various accessions of the plant species were cut and pasted into the programme (Translater), and by translating in 3 reading frames, the DNA (base nucleotides that code for the specific amino acids in the protein domain) for the various domains were recorded as shown in Tables 6.5a and b; and 6.6a and b).

6.3.2.1.1 Primers design for starch phosphorylase

The forward primer for the starch phosphorylase enzyme, STPH IF, was designed using the domains GRLASCF that occurs at position 1 of the total alignment length of the domains conserved for starch phosphorylase (Table 6.5a). Comparing the base nucleotides that code for the specific amino acids in the protein of the domains across the various plant species, the forward primer, STPH 1F was obtained as: 5'GGNAGBCTHGCHTCNTGYTT (Tm = 62.9^{o}C, Mol. Wt = 643 µg), where N = A, C, G, T; B = A, G; H = C, A, T; and Y = C, T (Table 6.5a). The reverse primer for the starch phosphorylase enzyme, STPH 564R, was designed from the domains, GGKAFATY which are located at position 564 of the total alignment. To get the STPH 564R, first the forward primer (STPH 564F) needed to be designed and the reverse

primer was simply obtained by reading the nucleotide backwards (reversing it). Thus comparing the nucleotides across the list of the plant species as shown in Table 6.5b, then, the forward primer of starch phosphorylase, that is primer STPH 564F, was thus obtained as: 5'GGWGGDAARGCDTTYGCNACNTAY, where W= A, G; D = A, G, T; B = A, G; Y = C, T; N = A, G, C, T (Table 6.5b). By reversing the above, the reverse primer, that is primer STPH 564R was therefore obtained as shown below: 5'YTANGTNGCYAADGCRTTDCCWCC (Tm = 64.7 ^{o}C, Mol. Wt = 943 µg).

Table 6.5: Conserved protein domain of starch phosphorylase enzyme

(a) Position 1 of the total alignment

Position 1	**G**	**R**	**L**	**A**	**S**	**C**	**F**
Ipomoea batata	GGA	AGG	CTT	GCT	TCT	TGC	TTT
Vitis vinifera	GGA	AGG	CTT	GCT	TCA	TGC	TTT
Vitis vinifera	GGA	AGG	CTT	GCT	TCC	TGC	TTC
Ricinus communis	GGA	AGA	CTT	GCT	TCA	TGC	TTT
Populus trichocarpa	GGA	AGA	CTT	GCT	TCA	TGT	TTT
Citrus hybrid cultivar	GGC	AGG	CTA	GCT	TCA	TGC	TTC
Cucurbita maxima	GGA	AGG	CTT	GCT	TCA	TGT	TTT
Arabidopsis thaliana	GGG	AGA	CTT	GCC	TCG	TGT	TTC
Arabidopsis thaliana	GGG	AGA	CTT	GCC	TCG	TGT	TTC
Arabidopsis thaliana	GGG	AGA	CTT	GCC	TCG	TGT	TTC
Arabidopsis thaliana	GGG	AGA	CTT	GCC	TCG	TGT	TTT
Vicia faba	GGT	AGG	CTT	GCT	TCT	TGC	TTT
Spinacia oleracea	GGG	AGG	CTC	GCT	TCG	TGC	TTT
Oryza sativa	GGT	AGG	CTC	GCA	TCT	TGC	TTT
Oryza sativa	GGT	AGG	CTC	GCA	TCT	TGC	TTT
Sorghum bicolar	GGT	AGG	CTT	GCA	TCT	TGC	TTT
Zea mays	GGT	AGG	CTT	GCA	TCT	TGC	TTT
Zea mays	GGT	AGG	CTT	GCA	TCT	TGC	TTT
Triticum aestivum	GGC	AGG	CTT	GCA	TCT	TGC	TTT

(b) Position 564 of the total alignment

Position 564	**G**	**G**	**K**	**A**	**F**	**A**	**T**	**Y**
Ipomoea batata	GGG	GGA	AAA	GCA	TTT	GCG	ACC	TAT
Vitis vinifera	GGA	GGA	AAA	GCA	TTC	GCT	ACA	TAC
Vitis vinifera	GGA	GGA	AAA	GCA	TTC	GCT	ACA	TAC
Ricinus communis	GGA	GGA	AAA	GCA	TTT	GCG	ACA	TAC
Populus trichocarpa	GGA	GGA	AAA	GCA	TTT	GCA	ACA	TAT
Citrus hybrid cultivar	GGA	GGG	AAA	GCA	TTT	GCA	ACA	TAT
Cucurbita maxima	GGA	GGA	AAA	GCA	TTT	GCT	ACA	TAT
Arabidopsis thaliana	GGG	GGT	AAA	GCA	TTT	GCC	ACC	TAT
Arabidopsis thaliana	GGG	GGT	AAA	GCA	TTT	GCC	ACC	TAT
Arabidopsis thaliana	GGG	GGT	AAA	GCA	TTT	GCC	ACC	TAT
Arabidopsis thaliana	GGG	GGT	AAA	GCA	TTT	GCC	ACC	TAT
Vicia faba	GGA	GGA	AAG	GCA	TTT	GCA	ACG	TAC
Spinacia oleracea	GGA	GGA	AAA	GCT	TTT	GCC	ACA	TAT
Oryza sativa	GGG	GGA	AAA	GCA	TTC	GCG	ACT	TAC
Oryza sativa	GGG	GGA	AAA	GCA	TTC	GCG	ACT	TAC
Sorghum bicolar	GGA	GGG	AAA	GCA	TTT	GCA	ACA	TAC
Zea mays	GGA	GGG	AAA	GCG	TTT	GCA	ACA	TAC
Zea mays	GGA	GGG	AAA	GCG	TTT	GCA	ACA	TAC
Triticum aestivum	GGA	GGG	AAA	GCA	TTT	GCA	ACA	TAC

6.3.2.1.2 Primers design for starch synthase

The forward primer of the starch synthase enzyme, SS 13F was designed from the domain KTGGLGDV, located at position 13 of the multiple alignment of the proteins conserved for starch synthase. Thus comparing the nucleotides specific for the domain KTGGLGDV, across the plant species, primer SS 13F of starch synthase was derived as: 5'AAAACAGGTGGBCTBGGWGATG (Tm = 57.7^{o}C, Mol. Wt = 489 µg), where, B = C, T or G; W = A or T (Table 6.6a). To get the reverse primer 109R, first there was the need to design the forward primer (i.e. primer 109F) from position 109 of the conserved domains, FCKAAVE. Therefore, comparing the nucleotides or bases specific for the domain FCKAAVE, which is located at position 109 of the conserved domains of starch synthase, the forward primer, that is 109F was obtained as: primer 109F = 5'TTRTGCAAYGCNGCWGTVGAG (Table 6.6b), where R= C, T; W= A, G; N= C, T, A, G; Y= A, T, G; V= C, T, and G. Then, reversing the above, primer 109 R of starch

synthase was obtained finally as: 5'CTCVACWGCNGCYTTGCARAA (Tm = 71^{o}C, Mol Wt = 553 µg).

Table 6.6: Conserved protein domain of starch synthase enzyme

(a) Position 13 of the total alignment

Position 13	**K**	**T**	**G**	**G**	**L**	**G**	**D**	**V**
Triticum aestivum	AAA	ACA	GGT	GGT	CTG	GGA	GAT	GTT
Triticum aestivum	AAA	ACA	GGT	GGT	CTT	GGA	GAT	GTT
Aegilops geniculata	*	*	GGT	GGT	CTG	GGA	GAT	GTT
Hordeum vulgare	AAA	ACA	GGT	GGT	CTT	GGA	GAT	GTT
Zea mays	AAA	ACA	GGT	GGT	CTT	GGA	GAT	GTT
Zea mays	AAA	ACA	GGT	GGT	CTT	GGA	GAT	GTT
Oryza sativa	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTT
Oryza sativa	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTT
Vitis vinifera	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTC
Manihot esculenta	AAA	ACA	GGT	GGC	CTT	GGT	GAT	GTC
Glycine max	AAA	ACA	GGT	GGT	CTT	GGA	GAT	GTT
Glycine max	AAA	ACA	GGT	GGT	CTT	GGA	GAT	GTT
Ricinus communis	AAA	ACA	GGT	GGC	CTC	GGA	GAT	GTT
Lotus aboriginus	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTT
Populus trichocarpa	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTT
Amaranthus cannabinus	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTT
Vigna unguiculata	AAA	ACA	GGT	GGG	CTT	GGT	GAT	GTA
Vigna unguiculata	AAA	ACA	GGT	GGG	CTT	GGT	GAT	GTA
Pisum sativum	AAA	ACA	GGC	GGG	CTT	GGA	GAT	GTT
Arabidopsis thaliana	AAA	ACA	GGT	GGC	CTT	GGA	GAT	GTA
Arabidopsis thaliana	AAA	ACA	GGT	GGC	CTT	GGA	GAT	GTA
Phaseolus vulgaris	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTA
Solanum tuberosum	AAA	ACA	GGT	GGG	CTT	GGA	GAT	GTT

Table 6.6: Conserved protein domain of starch synthase enzyme

(b) Position 109 of the total alignment

position 109	**F**	**C**	**K**	**A**	**A**	**V**	**E**
Triticum aestivum	TTC	TGC	AAG	GCC	GCT	GTT	GAG
Triticum aestivum	TTC	TGC	AAG	GCC	GCT	GTC	GAG
Aegilops geniculata	TTC	TGC	AAG	GCC	GCT	GTT	GAG
Hordeum vulgare	TTC	TGC	AAG	GCC	GCT	GTC	GAG
Zea mays	TTT	TGC	AAG	GTT	GCT	GTT	GAG
Zea mays	TTT	TGC	AAG	GTT	GCT	GTT	GAG
Zea mays	TTT	TGC	AAG	GTT	GCT	GTT	GAG
Oryza sativa	TTT	TGT	AAG	GCT	GCT	GTT	GAG
Oryza sativa	TTT	TGT	AAG	GCT	GCT	GTT	GAG
Oryza sativa	TTT	TGT	AAG	GCT	GCT	GTT	GAG
Vitis vinifera	TTT	TGC	AAG	GCA	GCT	ATT	GAG
Manihot esculenta	TTT	TGC	AAA	GCT	GCT	GTT	GAG
Glycine max	TTT	TGC	AAG	GCA	GCT	GTT	GAG
Glycine max	TTT	TGC	AAG	GCA	GCT	GTT	GAG
Ricinus communis	TTT	TGC	AAA	GCA	GCT	ATT	GAG
Lotus aboriginus	TTT	TGC	AAG	GCA	GCT	GTC	GAG
Populus trichocarpa	TTT	TGC	AAA	GCA	GCT	GTT	GAG
Amaranthus cannabinus	TTC	TGT	AAG	*	GCA	GTT	GAG
Vigna unguiculata	TTT	TGC	AAG	GCA	GCA	GTG	GAG
Vigna unguiculata	TTT	TGC	AAG	GCA	GCG	GTT	GAG
Pisum sativum	TTT	TGC	AAG	GCG	GCG	GTT	GAG
Arabidopsis thaliana	TTT	TGC	AAG	GCT	GCT	GTT	GAG
Arabidopsis thaliana	TTT	TGC	AAG	GCT	GCT	GTT	GAG
Phaseolus vulgaris	TTT	TGC	AAG	GCA	GCA	GTT	GAG
Solanum tuberosum	TTT	TGC	AAA	GCA	GCG	ATT	GAG

6.3.2.2 PCR reactions

Two PCR reactions were carried out in 2009/10. The first reaction (reaction 1), involved the use of the primers designed for the starch phosphorylase enzyme (STPH 1F and STPH 564R) whilst the second reaction (reaction 2), made use of the primers designed for the starch synthase enzyme (SS 13F and SS 109R). In each case, the PCR reaction mixture was made up of the same composition as that of year 2008, except that in place of the primers used in that year, 1 µl each of the forward and reverse primers designed for year 2009 for the respective enzymes were used. The PCR cycling conditions remained the same as those of year 2008. The annealing temperature for reaction 1 was 55 ^{o}C, and at this temperature, two PCR bands, one being the required band of about

563 bp, were obtained (Fig 6.6). The DNA fragments were isolated from the band containing the PCR product, as already described, prior to cloning. Reaction 2 was maintained at annealing temperature of 50 ^{o}C, producing a PCR product (band) of about 96 bp as anticipated, and the DNA fragments from this PCR product were also isolated prior to cloning.

6.3.2.3 Colony purification of bacterial strains

White colonies from reactions 1 and 2 were involved in PCR reactions using the same reaction conditions and the original primers, but the number of cycles was reduced from 35 to 15. The colonies that produced the right band size were thus identified. Other PCR reactions, involving the use of M13 primers instead of the original primers, were also carried out, using colonies identified from this reaction. The PCR products produced from reactions with the original primers were compared with the bands produced with the M13, and the samples that gave the right size of band from the two sets of primers (original primers and the M13) were noted (see Fig 6.7). The PCR samples of reaction 1 containing the M13 primers, which yielded the required band size (563 bp) were purified using the PCR purification kit as detailed above, prior to sequencing. The *E. coli* plasmid miniprep DNA preparation was conducted on colonies identified from reaction 2.

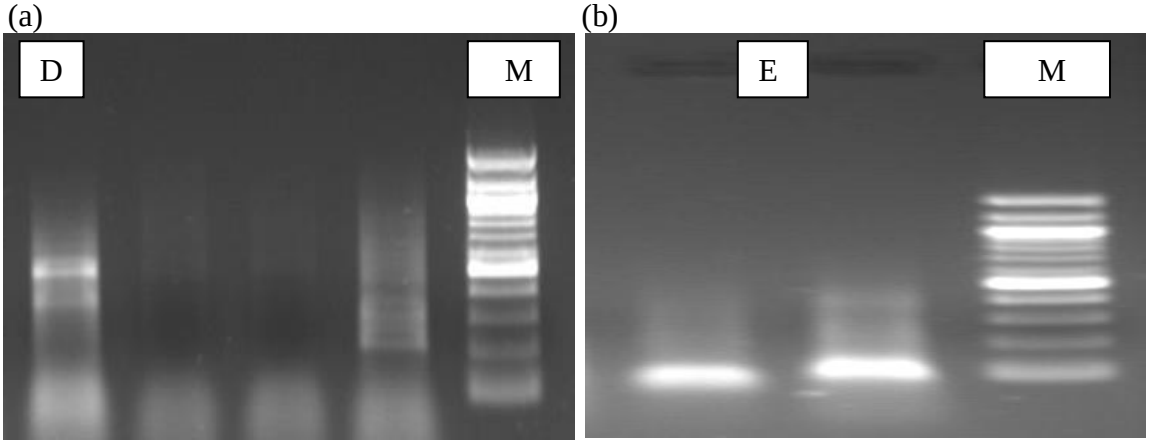
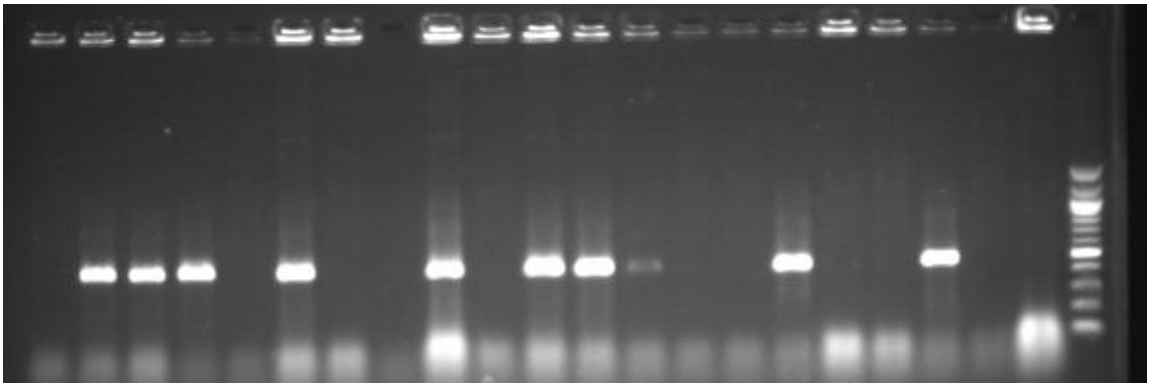
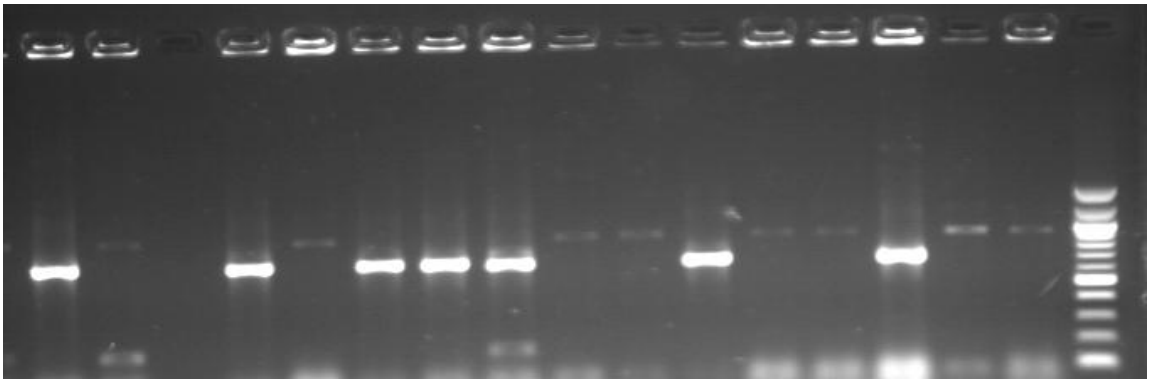


Fig 6.6: PCR product (a) D (563 bp), and (b) E (96 bp), obtained by using the starch phosphorylase and starch synthase primers, respectively. M represents the 100 bp marker used.

(a)



(b)



(c)

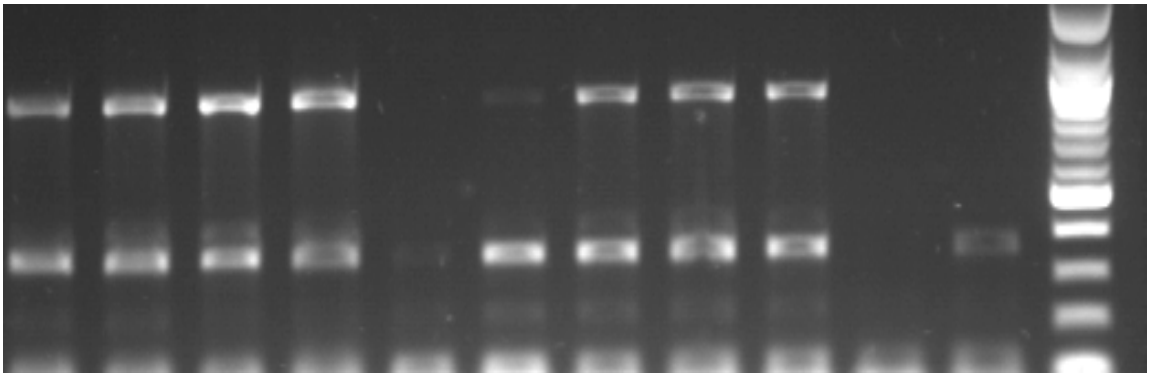


Fig 6.7: PCR products involving (a) clones of starch phosphorylase with the original primers, (b) PCR of starch phosphorylase clones with M13 primers, and (c) pcr of starch synthase with M13 primers; M, the 100 bp marker, is found at the extreme right of each photograph.

(a)
GGGAGGCTTGCATCGTGCTTGAAGAAAAGGTGAAGATTTTCACTGATCATA
AAAGCTTGAGGGTTATGGATTGAGGTACAAACATGGACTGTTCAAGCAACG
TATCACCAAAGCAGGACAAGAGGAGATTGCTGAAGATTGGCTGGAGAAATT
CAGTCCCTGGGAAGTTGCAAGGCATGATGAGCAGACTTCCAGCAGTGATGC
TTCTTACCTCACAGAGCAGACTTCAGCAGAGCTTGTGCGGGATGGACATTG
AGGTTATTCTTTCAGGAGAGTCAGCTTCACTGATGAGTTTGCAGATTCAGTC
GACTTTAGTGGATCGGATCAAGGCTGGACAAGCTGGAGATCCGAGATTGCA
GAAGATCAGAGCAGAGGTTCTAGCAGGCCAGAGACCAGAGTTTCAGATTCA
CGAGGATGGATCGTTGCGCTATGGAGTCAGATTGTGTGTTCCTTCTGGTGAC
ATCAGAGATGAGCTTTTGTTGGAGGCAAGGCATTCGCAACATATA

(b)
CATGCACCGTGTAGTAGCTGATATCACGGTTGAACGTGGTCGTCGTGGCTGC
TGAGTGTTCTCCCTGGTG CAAAACAGGTGGTCTGGGAGTGGCGGTGTACA

Fig 6.8: Sequencing results of the clones identified from reactions (a) 1 and, (b) 2 in year 2009/10, that is the sequences for starch phosphorylase and starch synthase, respectively.

6.3.2.4 The BLAST search

Sequences generated in year 2008 (Fig 6.5) using BLAST search, did not have any similarity with anything, according to the search but in 2009/10, the two sequences generated for starch phosphorylase and starch synthase (Fig 6.8) were similar to the sequences of these enzymes in sweet potato and sorghum, respectively. Figure 6.9 shows the sequence alignments of these enzymes as found in hyacinth and the other plant species.

(a)

Sweet potato STPH	62	GYGLRYKHGLFKQRITKAGQEEIAEDWLEKFSPWEVARHD	181
		GYGLRYKHGLFKQRITKAGQEEIAEDWLEKFSPWEVARHD	
Hyacinth STPH	21	GYGLRYKHGLFKQRITKAGQEEIAEDWLEKFSPWEVARHD	60

(b)

Sorghum bicolor SS	30	LNVVVVAAECSPWCKTGGLGVAV	98
		+NV+VVAAECSPWCKTGGLG V	
Hyacinth SS	252	MNVIVVAAECSPWCKTGGLGDVV	274

Fig 6.9: Sequence alignments of (a) Hyacinth starch phosphorylase (STPH) with sweet potato starch phosphorylase, and (b) Hyacinth starch synthase (SS) with the starch synthase of *Sorghum bicolour*. Sequences were generated using the blastx on the nr database (NCBI). Identities are highlighted in red colour.

6.4 Discussion

Sequences generated during the experimental period of year 2008 did not have any similarity with the genomic sequences of the enzymes (i.e. ADP glucose pyrophosphorylase, starch phosphorylase, starch branching enzyme and starch synthase) under consideration, and indeed, the sequences were not similar to anything. Maybe, those sequences were generated as a result of the primers hybridising to other genomic sequences (Schmalenberger *et al*., 2001). However, the sequences generated for starch phosphorylase and starch synthase from hyacinth, during the experimental period of year 2009/10 were similar to the sequences of these enzymes from a number of plants. As for example, the hyacinth starch phosphorylase sequence produced from the present study was similar to the starch phosphorylase sequences from Sweet potato (*Ipomoea batata*), *Ricinus communis*, *Arabidopsis thaliana*, *Citrus*, *Vitis vinifera*, *Oryza sativa*, *Triticum aestivum*, *Vitis vinifera* and maize (*Zea mays*), but that of sweet potato was the closest in similarity, according to the BLAST search. The observed similarity of the hyacinth starch phosphorylase sequence (produced from this study) to that of sweet potato and the other plant species is due to the fact that, it was from these plants species that the conserved domains (Kapitononov and Yu, 1999) were selected during the design of primers for this enzyme (see Table 6.5a and b). The alignment of the hyacinth starch phosphorylase sequence with that of sweet potato is as shown in Figure 6.9a. In fact, the hyacinth starch phosphorylase sequence (Hyacinth STPH) generated in the present study (Fig 6.9a) is identical at the protein level to that of sweet potato. This is not surprising, as the sequence is well conserved. This sequence could be used to redesign primers which could have been used to get a bigger part of the gene from hyacinth DNA. Similarly, the insert could have been used to probe a hyacinth genomic library to get the whole gene. Additionally, this sequence could be used to design a primer which could be used for RT-PCR analysis.

As in the case of the hyacinth starch phosphorylase sequence, the sequence generated for starch synthase in this study was similar to the starch synthase sequences from a number of plant species from which the primers for this enzyme were designed: *Sorghum bicolor, Aegilops tauschii, Hordeum vulgare, Zea mays, Triticum aestivum, Oryza sativa, Amaranthus cruentus*. The starch synthase sequence from *Sorghum bicolor* was the closest in terms of similarity to the starch synthase sequence produced from hyacinth, according to the BLAST search, and the alignment of the latter with the

former is as shown in Figure 6.9b. In general, even though this sequence definitely corresponds to starch synthase, it is clearly a short segment. Some of it may be from the primer, but there is a bit which is also from hyacinth. The sequence (Fig 6.9b) could be used to make a specific primer with a view to isolating more of the gene from hyacinth DNA. Alternatively, as mentioned in the case of the starch phosphorylase sequence, the insert could be used to probe a hyacinth genomic library to get the whole gene (Lutz, 2003). Designing a specific primer rather than a degenerate one would have a much greater chance of success so even a short sequence which contains no degeneracy will be much more successful.

Fragments of two of the enzymes, starch phosphorylase and starch synthase have been isolated from hyacinth in the present study. However, some difficulties were encountered during the design of the primers because there were virtually no close relatives of hyacinth in the databases, thus conserved domains of a number of plant species ranging from monocotyledonous species to dicotyledonous ones were used. Also, during experimentation, particularly in carrying out the PCR reactions, it was difficult for degenerate primers to prime specifically, that is, a high annealing temperature did not produce any PCR product, and too low an annealing temperature gave rise to multiple bands (mixed priming).

Studies in the future on this area of research could be possible, because the sequences generated for starch phosphorylase and starch synthase can be used to design new primers which are specific to hyacinth. This means that there will be much greater chances of getting more specific products than the present study. Full characterisation of genes can be made by making and probing genomic libraries and isolating clones from cDNA libraries. The gene expression of these enzymes can be studied by using specific probes for RT-PCR analysis (Tangphatsornruang *et al*., 2005).

CHAPTER 7

GENERAL DISCUSSION

7.1 Aim of the thesis

Flower bulbs are important ornamental geophytes grown mainly for the production of cut flowers, as potted plants for indoor or outdoor decorations, or as landscaping plants. In addition, because hyacinth flowers have a strong sweet fragrance, they are used in the perfumery industry for the manufacture of perfumes and oils. In spite of the importance of flower bulbs, little is known about their biochemistry, particularly in relation to the regulation of carbohydrate partitioning and metabolism, and the general aspects of their growth and physiology. For instance, the biochemical principles underlining the synthesis and/ or breakdown of carbohydrates in hyacinth, or the lily are poorly understood. In general, geophytes have the ability to store and remobilise reserved metabolites particularly carbohydrates, and the metabolism of the reserved carbohydrates in these species is essential to their growth and development. In recent years, the interest in the genes involved in the biosynthesis and degradation of carbohydrates, particularly starch, has increased because the genes involved in carbohydrate regulation and metabolism in plants provide them with important mechanisms for adjusting to various environmental changes. The present study was therefore conducted, to investigate the changes in the concentrations of carbohydrates, especially starch of the various organs of hyacinth or the lily during the growth of the bulb, either in relation to the utilization of the reserves in the mother bulb, or their accumulation in the newly formed organs. The study also sought to determine the relationships between starch and sugars, and the distribution of activities of enzymes that are involved in starch metabolism within these bulbous plants, at various stages of their growth. In general, the process of carbohydrate metabolism is influenced by the gene expression of enzymes that play a major role in that process. Studies at the molecular level were initiated with a view to generating sequence information from enzymes that are involved in the synthesis and degradation of starch in hyacinth. This information will enhance an investigation into the expression of starch metabolism genes in the test plant by using specific probes for RT-PCR. Plant growth and development is influenced by both biotic and abiotic factors. The relationships between assimilates supply and demand is best studied when the sources of assimilates are removed. Bulbs of the common hyacinth and the lily were therefore subjected to simulated herbivory and drought stress, with a view to understanding the physiological mechanisms of these species to cope with herbivory and water stress. In plant production, problems that reduce the yield and quality of products can be controlled if

the grower embarks on good agronomic practices. The present study sought to determine the effects of some agronomic practices such as nutrients supply, depth of planting, bulb size at planting and varieties on growth and development of the bulbs.

7.2 Carbohydrates partitioning and metabolism

Starch content and biomass of the bulb scales of hyacinth, and those of the lily decreased after planting, whilst values of these parameters of the newly formed organs (flower, new leaves and stem and roots) generally increased. The observed decrease in these parameters of the bulb scales following planting, was as a result of the formation and development of roots and shoots (sprouting), which occurred at the expense of the reserves deposited in the scales during the previous season (Du Toit *et al*., 2004; Ohyama *et al*., 1998; Matsuo and Mizuno, 1974). This is because, in hyacinth, the reduction in starch and biomass was highest at 1-2 months after planting (Fig 3.02a, Table 3.2a), coinciding with the time of sprouting or emergence of the bulb. It has already been established in Chapter 4 that, unpeeled hyacinth bulbs (control) took on average 51 days to emerge (Fig 4.02a) from the soil after planting, whilst peeled bulbs sprouted 59 days on average after planting. The period of sprouting for the unpeeled bulbs (i.e. 51 days after planting) is the same period (1-2 months after planting) where starch degradation was at its peak in Chapter 3. The implication of these findings is that, in flowering bulbs, the period where the rate of starch depletion is high in the bulb scales is the time where sprouting occurs. It also means that for emergence to occur, the developing plant will have to make use of the reserves stored in the bulb scales (Theron and Jacobs, 1996, Orthen, 2001). Even after emergence, the starch content and biomass of the scales continued to drop up to 4-5 months after planting (Fig 3.02a, 3.02a and 3.04b), whilst the values of these parameters increased in the newly formed organs because the developing plant made use of the reserves or the energy stored in the mother bulb to grow. This is in accordance with the observation that in geophytes, the utilisation of stored reserves is essential for the initial growth of the developing plant (Miller, 1992). The accumulation of starch and biomass of the newly formed organs particularly those of the flower, following the depletion of reserves of the scales, particularly the outer scales before current photosynthesis implies that, in flower bulbs, there exists an export of nutrients (reserved carbohydrates from the scales, especially from the outermost scale) to the newly formed organs. Thus at the early stages of

growth when no photosynthesis took place, it was this export of reserves from the scales that enhanced the growth of the flower, the new leaves, and the stem and roots. In the case of hyacinth, following the depletion of reserves in the bulb scales, the flower accumulated the highest amount of starch and biomass, whilst in the case of the lily; it was the stem and roots that accumulated the highest amount of these quantities. Therefore in bulbous plants, like many other geophytes, the importance of this export of materials or nutrients (reserve carbohydrates) from the bulb scales to the newly formed organs cannot be overemphasized. The outer scales provided the highest amounts of nutrients or reserves relative to that of the inner scales. This observation is in agreement with the finding made by Chen (1969) who also reported that in bulbs of *Narcissus tazetta*, the outer scales lost more reserves than the inner scales. Thus, it is due to this relationship or connection that exists between the scale leaves of bulbous plants and the flower, that any time the scales were removed or peeled away (Chapter 4), flower production or quality was affected. It means that, the removal of scales (peeling) reduces or stops the export of nutrients or reserves from the scales to the flower, hence the detrimental effects of scale removal on flower quality (Chapter 4). The same reason is responsible for the reduction in chlorophyll content of plants produced from bulbs whose scales were removed prior to planting. According to Miller *et al*., (1997), the scales of geophytes are not only reservoirs of storage carbohydrates, proteins or water, but also mineral salts and ions. The removal of bulb scales as a result of peeling (Chapter 4) either inhibits or reduces the concentration of nutrients and/or ions, notably Mg^{2+}, which are needed for chlorophyll synthesis, and their export from the scales to the leaves. The general accumulation of starch in the scales from 5 to 7 months after planting is an indication of the contribution from current photosynthesis. The rate of depletion of starch and biomass of the scales was higher in the outermost scales (L1-L4) than the inner scales (L5-L7); and in fact, scales L1-L4 behaved similarly, whilst L5-L7 also performed similar metabolic functions and this was the basis of grouping the scales together for the subsequent studies during the 2008/2009 seasons. The generally low concentrations of sugars in the scales of hyacinth as compared to that of starch especially before planting, coupled with the disappearance of the sugars from the scales during senescence (Chen, 1969; Vishnevetsky *et al*., 2000), and the accumulation of starch in the scales from 5-7 months after planting (before harvesting) is an indication that starch was the dominant storage carbohydrate in hyacinth.

Carbohydrate metabolism in the case of the lily bulb was slightly different from that of hyacinth, in that for the former, both glucose and sucrose, together with starch of the bulb scales were metabolised during the early developmental stages and this provided energy to fuel the emergence of shoots and roots during the first 3 weeks after planting. Fructose, however, accumulated in the scales at 0-3 weeks after planting. This means that, in the lily bulb, whereas sucrose and glucose were metabolised just like starch prior to sprouting, there was an inter-conversion of starch or glucose to fructose. Thus the metabolism of sucrose and glucose as well as starch in the scales of the lily was responsible for the development of the flower and the accumulation of the soluble sugars mainly glucose and sucrose enhanced the growth of the flower.

Results on the growth of hyacinth bulbs in either soil or glass tubes indicated that bulbs planted in glass tubes recorded lower percentage reductions in biomass and starch than those planted in the soil. This observation implies that planting in the glass tubes offered the opportunity to minimise the high reductions in starch and biomass that characterised the early stages of development of the bulbs when they were planted in the soil. During experimentation, it was observed that, early development of roots and shoots characterised planting in the soil and this is in accordance with the finding made by Nowak *et al*. (1974) that starch degradation and rate of metabolism of rooted plants occurred faster than the unrooted ones. In the present study, roots developed as early as 4 days after planting when bulbs were planted in the soil, and by 20 days after planting, leaves had started to develop from bulbs that were planted in the soil, whilst roots and shoots emergence was delayed when bulbs were planted in glass tubes. Thus it follows that whilst rapid metabolism of bulbs' reserves occurred for the bulbs that were planted in the soil, the rate of degradation or depletion of stored carbohydrates was lowered when bulbs were planted in glass tubes. Even though, the aim of the present study was not about the uptake of nutrients by the root system of this flower bulb in glass tubes (hydroponics), it is generally known that planting in glass tubes (hydroponics) leads to a more efficient utilisation of nutrients than planting in the soil (Cannabis Hydroponics, 2010). It is therefore due to this efficiency in the utilisation of nutrients of plants grown in hydroponics systems, coupled with the delay in roots and shoots formation that characterised the bulbs planted in glass tubes that were responsible for the lower reduction in starch when bulbs were planted in glass tubes, as compared to planting in the soil. In general, the high rate of development of roots and shoots (sprouting) of the

bulbs planted in the soil, as compared to those that were planted in glass tubes means that the activities of α-amylase and/or starch phosphorylase must have been higher in the case of bulbs that were planted in the soil than those planted in the glass tubes. According to Nowak *et al.* (1974), the activities of starch degrading enzymes were higher in rooted plants than the unrooted. Results on the measurement of α-amylase and starch phosphorylase in the bulb scales of hyacinth indicated that, the distribution of these enzymes in the flower bulb mimicked the distribution pattern exhibited by starch degradation in the scales. That is, the activities of α-amylase and starch phosphorylase were higher in the outer scales than the inner scales, indicating that starch degradation was higher in the former than the latter. Similarly, the relationship between the starch content of the outer scales and α-amylase activity was high ($r^2 = 0.6331$ or $r^2 = 0.7576$, Fig 3.12a and b), whilst the correlation was low ($r^2 = 0.3655$ or $r^2 = 0.4077$, Fig 3.12) for the enzyme activity and starch content of the inner scales. The activity of α-amylase increased from planting (September) to two months after planting (November) whilst that of starch phosphorylase increased from planting to 1 month after planting (October). This implies that, in general, at 1-2 months after planting, the activities of α-amylase and starch phosphorylase were high, and this coincides with the period at which sprouting of the unpeeled (control) bulbs occurred (Chapter 4). It means therefore that, sprouting in flower bulbs marks the period at which starch degradation is at its peak, and also, the activity of enzymes responsible for starch degradation is high during this time. This observation is thus in support of the finding made by Akazawa and Hara-Nishimura (1985). According to these authors, the activity of starch degrading enzymes as well as starch degradation increases at the time of sprouting in most geophytes. The correlation coefficient between the starch content of the scales, particularly the outer scales and α-amylase activity was higher ($r^2 = 0.6331$, or $r^2 = 0.7576$, Fig 3.12a and b), than that between starch phosphorylase and starch content of the scales ($r^2 = 0.011$, or $r^2 = 0.048$, Fig 3.13a and b). This shows that α-amylase predicted starch degradation in hyacinth more than did starch phosphorylase. In other words, starch degradation in the common hyacinth is more of amylolysis than phosphorolysis. In general, the activities of the two enzymes decreased 3 months after planting, because at this point, starch degradation was low. The increase in the activities of the starch synthesizing enzymes (ADP glucose pyrophosphorylase and starch synthase) of the newly formed organs particularly the flower, 1-4 months after planting indicates the accumulation of starch in these organs, and this followed the pattern of starch content in the organs. The activities

of the two starch synthesizing enzymes correlated better with starch accumulation of the flower and the newly formed leaves (Fig 3.14 and 3.15) than that of the stem and roots, however, the activities of the two enzymes were similar, implying that ADP glucose pyrophosphorylase and starch synthase were equally responsible for the accumulation of starch in the flower as well as the newly formed leaves but they were less responsible for starch accumulation in the roots and stem.

7.3 Herbivory

7.3.1 Below-ground herbivory

Bulbs peeled prior to planting took longer to sprout as compared to the unpeeled control. Bulbs, like many other geophytes, store a number of carbohydrates reserves such as starch, soluble sugars, glucomannans and fructans; proteins, mineral salts and water in their storage organs (Miller *et al.*, 1997). These substances are utilised by the geophytes to sprout when environmental conditions become favourable. The removal of bulb scales prior to planting therefore implies that not only the food reserves stored in the bulb scales were reduced, but also water, and even mineral salts were reduced in proportion to the volume of scales peeled away prior to emergence. In fact, it has already been emphasized in section 7.2, and also in Chapter 3 that the period at which sprouting occurred in bulbs, coincide with the time where the degradation of carbohydrate (particularly starch) is at its peak. Also, the activities of α-amylase and starch phosphorylase are high during sprouting of bulbs. All these observations suggest that in flower bulbs, sprouting utilises a lot of the reserves stored during the preceding season. It means that, peeling was very devastating right from the onset, to the initial growth and development of the bulbs, because it denied the developing plant the energy needed for early sprouting, and this reflected also in the growth of these plants. The developing plants needed the resources stored in the bulbs not only for sprouting (emergence), but also for their initial growth and development (Wassink, 1965) since at this time, the plants lacked photosynthetic leaves to carry out the essential process of photosynthesis. Thus, hyacinth and lily plants produced from bulbs whose scale leaves were peeled before planting did not only show a delay in emergence, but they also experienced a reduction in vegetative growth. The reduction in growth of plants produced from peeled bulbs implies that, their mother-bulb food reserves as well as the general photosynthetic apparatus were affected. Consequently, these plants also

exhibited a reduction in photosynthetic rates, which might have adversely influenced dry matter production and partitioning of their organs. The severer the scale removal treatment, the higher the interference and damage caused to the mother-bulb food reserves and the photosynthetic apparatus of the plants (Rees, 1971; Kim *et al*., 2003) and therefore, plants produced as a result of complete scale removal recorded a higher reduction in growth, rates of photosynthesis and stomatal conductance than those subjected to the partial scales removal treatments. Consequently, plants produced from peeled bulbs also had low bulb yield at harvest relative to those of the unpeeled control. Thus the behaviour of plants produced from peeled bulbs was similar to those plants produced from small bulbs (Chapter 5). However, in the case of the lily, plants produced from complete bulb scales removal prior to planting recorded a higher bulb yield at harvest (weight gain) than those from the unpeeled control, a situation that was least expected. Perhaps, in the lily, the absence of flowering following complete scale removal, might have redirected assimilates produced from photosynthesis to the bulb either for the development of this organ or for storage. Die *et al*. (1970) also reported that in flowering bulbs, the flower was an important sink organ for photosynthates. However, in hyacinth, the unpeeled control produced better bulb yield than plants produced from peeled bulbs. The observed difference between the two species in terms of bulb yield at harvest as a result of peeling, among other factors may be as a result of genetic differences between the two species.

The influence of bulb scale removal on chlorophyll formation cannot be overemphasized. The scale removal treatments dramatically reduced the chlorophyll concentration of plants as compared to plants produced from the unpeeled bulbs. Thus, apart from denying the developing plants of reserve food substances and water, peeling also reduced the concentrations of ions necessary for the synthesis of chlorophyll. In fact, in terms of chemical structure, the chlorophyll pigment is a chlorin ring with Mg^{2+} ions at the centre of the ring. In general, because plants produced from peeled bulbs recorded lower chlorophyll concentration than those from the unpeeled bulbs, it suggests that, the bulb is not only a reservoir of food substances or water, but also a storage sites for components of chlorophyll synthesis, particularly, ions such as Mg^{2+}, which are needed for chlorophyll synthesis. Thus the removal of the bulb scales also means the removal of ions (Miller *et al*., 1997) that are essential for the synthesis of chlorophyll. Plants produced from complete bulb scale removal recorded lower

chlorophyll concentrations than those from the partial scale removal treatments because in the case of the former, virtually all these ions, or at least, more of the ions were removed as compared to the latter. Similarly, plants produced from bulbs whose scales were removed at two weeks after planting recorded higher chlorophyll content than plants whose bulb scales were removed prior to planting. It has already been reported (Chapter 4) that plants produced from peeled bulbs prior to planting delayed in emergence relative to those from the unpeeled control. The delay in emergence also implies that there was a delay in their roots development. This indicates that plants produced from peeled bulbs prior to planting were placed at a competitive disadvantage in terms of water and minerals absorption from the soil (Caldwell *et al.*, 1987) as compared to the unpeeled control and those whose bulb scales were peeled two weeks after planting. Plants whose bulbs scales were peeled two weeks after planting had already developed roots system for water and minerals uptake before peeling was carried out. The poor minerals and water uptake of the plants whose bulbs scales were removed prior to planting (because of a delay in roots formation) as compared to those whose bulb scales were peeled two weeks after planting explains the reduction in chlorophyll concentration of the former as compared to the latter.

In both hyacinth and the lily, complete bulb scales removal inhibited flowering, whilst partial scales removal reduced flower quality. Studies on carbohydrate metabolism (Chapter 3) produced evidence that, at the early stages of growth, the growth of the flower depended on the reserves stored in the bulb scales (Bowen and Pate, 1993). Thus the degradation or depletion of these reserves, mainly starch of the scales, led to their accumulation in the newly formed organs particularly the flower. This was supported by the increase in the activities of α-amylase and starch phosphorylase in the scales, particularly the outer scales, during the first three months after planting of the bulb, whilst the activities of the starch synthesizing enzymes, ADP glucose pyrophosphorylase and starch synthase of the flower increased. This clearly demonstrates the importance of the reserves stored in the bulbs to flowering in bulbous plants. Therefore, in bulbous plants, flowering appears to be intimately linked to the reserves of the planted bulb, rather than the leaf growth produced. Thus, immature or small bulbs displayed little or no flowering (Chapter 5) until a specific bulb size is attained. The implication here is that traumatic bulb damage during dormancy will lead

to either failure of flowering, or abortion of flowers during the growing period of the bulb, and the bulb devoting its growth to establishing flowering sized bulb.

7.3.2 Above-ground herbivory

In hyacinth, plants did not compensate for leaf loss following defoliation because growth was always reduced, whilst flowering was not affected. This means that once leaves are above ground, any minor or devastating damage to the leaves will not stop the plants from flowering, flowering will continue as would have occurred without the damage. This contrasts with scale leaf damage of this flower bulb. In fact, both complete and partial leaf removal in hyacinth were detrimental to the growth and subsequent development of the bulbs, but plants from the former regime suffered more reductions in growth than those from the latter regime, and this reflected also in their reduced bulb yield at harvest as compared to the control. In fact, the plants whose leaves were defoliated had reduced sizes of the leaf canopy with a concomitant effect on photoassimilates production. Defoliated plants might have also suffered a reduction in carbohydrate reserves through respiration and regrowth (Carlson, 1966). Similarly, a reduction in root growth might have occurred and leaves were produced at the expense of the root system. This implies that, in hyacinth, the defoliated plants were placed at a competitive disadvantage position as compared to the control whose leaves were not removed, in terms of their ability to acquire resources for growth. According to Rockwood and Lobstein (1994), during regrowth after defoliation, energy is invested in refoliation and is unavailable for growth and maintenance. Therefore, defoliated plants from hyacinth used their food reserves for vegetative growth instead of the reserves being used productively to enhance bulb yield, hence the reduction in bulb fresh weight of the defoliated plants at harvest. The decrease in bulb fresh weight at harvest of the defoliated plants as compared to that of the undefoliated control implies that hyacinth is very sensitive to reductions in assimilates supply.

However, in the case of the lily, plants responded positively to complete herbivory by compensating for leaf loss especially when the damage was exerted at the beginning of their growth. Physiologically, some adjustments must have taken place in these plants which allowed them to overcome the loss of the photosynthetic tissues. Probably, there was a reallocation of stored food reserves for the production of new but longer leaves

and this reflected in plants from this regime having 'unusually' very long leaves (picture shown i.e. Fig 4.20b) whose total leaf area were similar to that of the undefoliated control. Gadd, Young and Palmer (2001) also reported a compensatory regrowth in *Accacia drepanolobium*. In general, plant growth and productivity is dependent not only on photosynthesis, but also on the integrated processes of allocation, accumulation and utilisation of photoassimilated carbon, which control the carbon budget of the plants. The lily plants subjected to complete shoot herbivory at the beginning of their growth had no option but to rely exclusively on the mobilisation of stored reserves, and later, on current photosynthesis once the first new leaves were produced. In addition, the relatively high chlorophyll content of the newly formed leaves, coupled with the high photosynthetic rates and stomatal conductance might have enhanced the high regrowth of vegetation in these plants.

In general, the data presented showed that leaf or shoot removal exerted at the beginning of the season (1st herbivory regimes) was less damaging to the plants than the same treatments which occurred later in the season (2nd herbivory regime). This is because plants subjected to the 1st leaf herbivory produced higher vegetative growth and bulb yield than those from the 2nd herbivory regimes. This means that plants' response to herbivory, among other factors, depend on the actual time, or the growth stage at which herbivory occurs (McNaughton, 1983; Rosenthal and Kotanen, 1994). Studies in Chapter 3 revealed that, the carbohydrates content of the bulb scales of the two species was higher at the time of 1st herbivory than that of the 2nd herbivory and therefore, it means that more resources (through remobilisation of carbohydrate reserves in the bulb) were available in the bulb scales to support growth and development during the time of the 1st herbivory than during the 2nd herbivory treatment.

7.4 Drought stress and bulbs scale removal

Drought negatively impacted on the growth and productivity of the two bulbous species under consideration. Plants subjected to drought stress did not only suffer a reduction in vegetative growth, but they also had decreased chlorophyll content, poor photosynthetic rates and stomatal conductance which ultimately reflected in their poor bulb yield at harvest as compared to those that received water application throughout the session. Plants react to drought by exhibiting some adaptive features such as increases in solute

concentration (Scott, 2008). In general, this mechanism causes an osmotic flow of water out of the cells of the plant and this leads to a reduction in water potential of the plant cells, membranes disruption and the occurrences of senescence and poor yield. The early flower abortion, wilting and shoot die back which are all indications of early senescence were observed in this study. Volaire (2002) also stated that, during water stress, leaf growth reduces, soil moisture declines, and the proportion of senescent tissues in total aerial biomass increased. The results of this study suggests that although geophytic plants like hyacinth and lilies survive dry periods as bulbs, both plants were very susceptible to drought stress in the growing phase of their life cycles. Therefore any adaptations bulbs possess to survive drought must be invested in the dormant bulb. The idea of scale removal in this work was to limit water availability to the plants. Thus plants produced from peeled bulbs of either hyacinth or the lily, which were also subjected to water stress suffered double stresses, that is the combined action of scale removal and drought stress were responsible for their poorest performance in terms of all parameters measured.

7.5 Bulbs size effects and depth of planting

7.5.1 Bulbs size effects

Studies in Chapter 4 on bulb herbivory revealed that scale removal had detrimental effects not only on growth, but also on flower production of the flower bulbs. The various peeling regimes reduced not only the sizes of the planted bulb, but also the amount of reserves stored, as would have been the case if small bulbs or bulbs of different sizes were planted. This prompted the research to investigate the influence of different bulb sizes at planting on growth and development of the species. In general, results indicated that, bulb size influenced vegetative growth, flower quality, bulb yield and bulblets formation, both in hyacinth and the lily. Plants obtained from the small bulbs behaved just like those produced from peeled bulbs (Chapter 4) because they had reduced vegetative growth, flower quality and bulb yield at harvest, as compared to plants produced from large bulbs. The observed decrease in the measured parameters of plants produced from small bulbs, as compared to those produced from large bulbs is due to the variation in the amount of stored reserves found in the bulbs at the time of planting (Rees, 1969; Burton, 1966; Watada *et al*., 1999). Under normal circumstances, the bigger the bulb, the higher the amounts of reserved carbohydrates stored in the bulb,

and since the initial growth of geophytes mainly depends on the remobilisation of the reserved metabolites stored in bulb particularly in the scales (Chapter 3), it is not surprising that plants produced from large bulbs recorded higher growth and development than those from the small bulbs. This shows that in geophytes or in flower bulbs, the performance of plants is influenced greatly by the amounts of reserves stored in the planting material at the time of planting. This is also because, in geophytes, large planting materials generally experience a higher rate of reduction in weight during emergence as compared to small planting materials, and the higher the reduction in weight during sprouting, the higher the growth rate (Hidekazu *et* al., 1998). In general, parameters measured increased with increasing bulb size at planting. But in the case of hyacinth, values of the measured parameters were similar after the 50 g bulb size. The observed increase in parameters with increases in bulb size at planting demonstrates the importance of reserves carbohydrates to growth and development of the flower bulb. However, in bulbous plants, the possession of too large a bulb seems to be of a little advantage, overall, to the plant in terms of vegetative growth and flower production as was observed in hyacinth, because bulbs of above 50 g sizes recorded similar values of the parameters measured. That is, after the 50 g bulb size, the results looked markedly similar. One will thus, question, the importance of the extra carbohydrate reserves of the plants obtained from large bulbs. Well, perhaps, the flower bulb uses this extra carbohydrate later during the life cycle of the bulb for survival during unfavourable environmental conditions (Miller *et al*., 1997). Working on *Brodiaea*, Han *et al.* (1991) observed that, growth and flower quality was independent of the mother corm size at the time of planting, but it was the size of the apical meristem that determined the quality of the flowers produced. Then, in the present study, another school of thought is, the flower bulbs uses the amount of reserves stored in the bulb prior to planting for its initial growth and development up to a certain threshold, or bulb size, in this case the 50 g bulb size. But above this size, growth and development may be determined by the size of the apical meristem. In this study, however, since measurements of the apical meristem was not made, the relationship between bulb size (carbohydrates reserves) and apical meristem in flower bulbs is not known, and this has not been documented in the literature.

In hyacinth, the chlorophyll concentration of plants produced from large bulbs was essentially higher than that of plants produced from small bulbs. However, values of

this parameter dropped drastically at 18 weeks after planting in the case of plants from large bulbs, whilst that of plants produced from small bulbs did not fall. A similar observation was made in the lily. Chlorophyll content of plants is a measure of photosynthetic rate or plant vigour. Therefore a fall in chlorophyll content could result in a decline in the essential process of photosynthesis. In the lily, the fall in chlorophyll content of plants produced from large bulbs was linearly related with the decrease in the rate of photosynthesis. However, a decline in chlorophyll content also marks the beginning of the onset of senescence in plants (Volaire, 2002). This was the case with plants produced from large bulb. Since plants obtained from large bulbs initially recorded higher chlorophyll content than plants produced from small bulbs, the former also recorded higher photosynthetic rate than the latter but senesced earlier than the latter. The manifestations of senescence in plants include a fall in chlorophyll content, and of course a reduction in the photosynthetic rate. Other symptoms of senescence in plants are flower and fruits abortion, shoots and leaf die back, wilting and death. All these observations occurred of plants from large bulbs, particularly in the lily, earlier than those from the small bulbs in this study. Thus in flower bulbs, planting from large bulbs will ensure better quality flowers and growth, but plants will grow and complete their life cycle earlier than those planted from small bulbs.

7.5.2 Depth of planting

Planting depth influenced greatly the growth and development of the bulbs, with the effects becoming very detrimental as the planting depths increased (Vogel, 1963). In general, the deeper the depth of planting, the longer it took the bulbs to emerge from the soil after planting. However, in hyacinth, bulbs planted at 5 cm depth emerged earlier than those planted at the 0 cm depth. Bulbs planted at 0 cm depth in the case of hyacinth lacked soil which is a good medium for germination or sprouting. Thus bulbs from this category delayed in roots and shoots emergence because of too much moisture loss from the bulbs as a result of evaporation. Results of starch metabolism of bulbs planted in glass tubes (Chapter 3) also confirmed that the rate of starch degradation of bulbs planted in glass tubes, during the initial phases of growth and development was low as compared to those planted in the soil. This is because, roots and shoots (of the developing plant) took longer to develop in the case of bulbs that were planted in glass tubes and since in flowering bulbs, like many other geophytes, the initial growth

depends on the amounts of reserves stored in the bulbs (Wassink, 1965), it means that the higher the rate of growth of shoots and roots, the higher the rate of depletion of the reserves. In addition, it has already been established that (Chapter 3), the period of highest carbohydrate breakdown in flower bulbs coincides with the time of sprouting (Akazawa and Hara-Nishimura, 1985; Lambrechts *et al*., 1994) and the time when the activities of α-amylase and starch phosphorylase are very high. Thus the absence of soil to cover the bulbs placed at the soil surface (0 cm depth) enhanced moisture loss from these bulbs which led to a delay in roots and shoots formation. It means that in the case of bulbs planted at 5 cm depth, because bulbs were covered to conserve moisture in the bulbs, sprouting occurred earlier in these bulbs and the activities of the hydrolytic enzymes might have been higher as compared to those planted just at the 0 cm depth, hence a delay in their emergence. However, the lily bulbs planted at the 0 cm depth did not sprout and this shows that the bulbs of this species could not withstand the loss of moisture from the bulbs; consequently, the enzymatic breakdown of starch to sugars to facilitate sprouting was not possible in this bulb. Hyacinth and the lily are both flower bulbs, but apart from the fact that hyacinth is a spring flowering bulb and lily is summer blooming, the two bulbous species might differ genetically.

Bulbs planted deep took longer to emerge from the soil as compared to those of shallow planting. The planting materials used in this experiment were of the same cultivar. This means that sprouting may have occurred at the same time, but because those planted deep were faced with the task of travelling longer distances than those of shallow planting for emergence to occur, the former emerged quite late as compared to the latter. In addition, bulbs planted deep might have expended a lot of their resources and energy to pushing their shoot upwards and so they emerged from the soil before growth and other developments occurred. This means that bulbs of deep planting regimes were placed at a competitive disadvantageous position in terms of acquiring resources (Louda *et al*., 1990) for growth and development. In any case, because plants were of the same cultivar, and should enter the rest period at the end of the growth season almost at the same time irrespective of planting depth, the growing season was shorter in deep planting than shallow planting. These reasons are responsible for the reductions in vegetative growth, flower quality, bulb fresh weight and bulblets formation as the depth of planting increased. Planting depth is therefore an important factor to consider in bulb production.

Towards the end of the growing season, plants produced from the deep planting regimes (15 cm and 20 cm depths) either in hyacinth or the lily, recorded relatively higher chlorophyll contents than those from the shallow planting (5 and 10 cm depth). That is, whereas the chlorophyll concentration of plants from the deep planting regimes was increasing during the growth season, those from the shallow planting regimes were declining. The decline in chlorophyll contents of plants, as already mentioned, is an indication of plant or leaf senescence (Volaire, 2002), and the manifestations of senescence in plants have already been discussed and will not be repeated here. Therefore in flower bulbs, the behaviour of plants produced from deep planting is similar to plants produced from small bulbs, because in these cases, there is a delay in senescence. On the contrary, plants from shallow planting, just like those produced from big bulbs, are characterised by high vegetative growth, and they produced high quality flowers and bulb yield at harvest, but they grew faster to complete their life cycle, thereby reaching senescence earlier as compared to those of deep planting.

At harvest, the depths at which the bulbs were located as compared to their original depths at the time of planting indicated that, in both hyacinth and the lily, the bulbs had made downward movements in the soil. This means that flower bulbs, like many other geophytes, struggle in their natural habitat to maintain their required depth, but by doing this, a number of their characteristics are affected (Galil, 1961). Results of this study show that the shallower the depth of planting, the greater the movement made by the bulbs at the end of the season. Shallow planting of bulbs, therefore, necessitates the bulbs to respond to these treatments by "pulling" themselves down into deeper ground. However, once plants are at an unfavourable depth in the soil (i.e. too deep) they seem to lack any mechanism for raising themselves in the soil profile. Maybe in their natural environment, weathering brings them closer to the surface, in a similar way as rocks and small stones rise to the surface. Results suggest that planting bulbs too deep in the soil can have major implications on plant productivity.

7.6 Bulbs and nutrients supply

In general, plants that received nutrients application from either ammonium sulphate [$(NH_4)_2SO_4$)] or dibasic sodium hydrogen phosphate (Na_2HPO_4) performed better than the control. It is already known that in flower bulbs, the reserves stored in the bulb

(mainly starch) decreased after planting particularly during the time of sprouting (Chapter 3). This reduction in carbohydrate reserves occurs because the starch degrading enzymes such as α- amylase and starch phosphorylase breakdown the reserved starch stored in the bulb into sugars which fuel the emergence and the subsequent growth and development of the developing plant. This means that, the amount of reserves stored in the bulb decreased following sprouting and growth of the bulb (Ohyama *et al*., 1998; Du Toit *et al*., 2004)). But at the initial stages of growth, the developing plants cannot photosynthesize because they lack mature or photosynthetic leaves to carry out the essential process of photosynthesis. Replacing the nutrients lost from the bulb during sprouting and the subsequent growth of the developing plant, through nutrients feeding further enhanced the growth and development of the bulbs and this was the case of the plants fed with nutrients, because in general, plants fed with nutrients had better growth and development than their control counterparts that received no nutrients application.

At the application rate of 60 or 90 mM of $(NH_4)_2SO_4$, both hyacinth and lily recorded higher vegetative growth and chlorophyll content than plants fed with Na_2HPO_4 and the control. The high chlorophyll concentration of these plants, coupled with their relatively high photosynthetic apparatus, might have enhanced dry matter accumulation in the bulb, which led to a higher fresh weight at harvest, not only of the mother bulb, but also the bulblets. The magnitude of parameters measured increased with increases in the rate of application of the nutrients. Ammonium sulphate produces nitrogen for plants uptake. Scott (2008) reported that nitrogen is so important, and plays a major role in plants that, its deficiency is detrimental to the growth and development of most plants. The nutrient element is also a constituent of some compounds such as chlorophyll, cell wall and nucleotides (Havlin *et al.*, 1999). Nitrogen is absorbed in the soil as NH_4^+ ions, but transformed in amine form and utilised to form enzymes and other proteins. The observed increase in growth and productivity of the flower bulbs in this study are therefore attributable to the fact that enzymes play a major role in a number of metabolic processes in plants.

On flower quality, the study showed that plants fed withNa_2HPO_4 performed better than the control and those fed with $(NH_4)_2SO_4$, in hyacinth. Na_2HPO_4 produces phosphorus for plants uptake in the soil. The observed increase in flower quality of hyacinth is due to the fact that phosphorus is a component of nucleic acids and genes, and plays a major

role in reproduction. In plants, phosphorus also enhances the production and maturation and quality of flowers and fruits (Bennett, 1993). In the lily, however, flower quality was not increased by feeding plants with Na_2HPO_4. It was rather, the plants that were fed with $(NH_4)_2SO_4$ that recorded an increase in flower quality. According to Treder (2000), variations in response of plants to nutrients application, among other factors is due to differences in their genotypic constitution. Therefore, the variation in the response to these nutrients, in terms of flower development of the two bulbous species, was as a result of differences in their genetic makeup. Results from Chapter 3 made it clear that the depletion of the reserves stored in the bulb scales led to the accumulation of these reserves notably starch, in the newly formed organs (the flower, stem and roots and newly formed leaves). This means that even in the absence of photosynthesis (during the early developmental stages of growth when no photosynthetic leaves had developed), the newly formed organs continued to grow and accumulate biomass and carbohydrates reserves at the expense of the reserves stored in the bulb scales (Miller, 1992). The reason for the observed difference in the response of hyacinth and the lily to nutrients feeding, especially in terms of their flower development apart from genetic reasons, can be deduced from the results from Chapter 3. The carbohydrates metabolism studies of the bulbs indicated that following planting and starch breakdown of the bulb scales, it was the flower that accumulated the greatest amount of biomass and starch in hyacinth, whilst in the case of the lily, it was the stem and roots that accumulated the highest amount of biomass and starch. This means that in hyacinth, the absorbed nutrients were used first in the development of the flower before the other organs, whilst in the case of the lily, the priority was given to the stem and roots development before the other organs. Plants fed with $(NH_4)_2SO_4$ generally recorded higher chlorophyll concentration than those that received nutrients application from Na_2HPO_4 and the control in both flower bulbs. In addition, the control and plants fed with Na_2HPO_4 recorded a relatively high reduction in chlorophyll as compared to those that received application from $(NH_4)_2SO_4$ towards the end of the season. Thus even at the time of harvesting, plants fed with $(NH_4)_2SO_4$ still looked greenish whilst the above-ground parts of plants from the other regimes had died back and completely senesced. This indicates that, just like the case of plants produced from small bulbs and those from the deep planting (15 and 20 cm) regimes, plants fed with $(NH_4)_2SO_4$ exhibited a delay in the rate of senescence as compared to the control and plants from the Na_2HPO_4. In the lily, plants fed with Na_2HPO_4 and the control were characterised by early abortion of

flowers, wilting and shoots die back as compared to plants fed with $(NH_4)_2SO_4$ and all these observations are in support of the fact that senescence delayed in the latter as compared with the former.

7.7 Varietal effects

Evaluation of the varieties for their bulb yield, flower quality and vegetative growth indicated that variety Sky Jacket recorded the highest vegetative growth, followed by Purple Voice, whilst Jon Bos and Pink Pearl produced low vegetative growth and biomass. The rest of the varieties recorded similar characteristics. Variations in the characteristics of these varieties may be due to differences in the genetic constitution (Troughton, 1970), or genotype x environment interactions (Mulder and Bijma, 2005). The relatively high vegetative biomass of Sky Jackets and Purple Voice implies that these two varieties had high photosynthetic apparatus. In addition, the high chlorophyll concentration of these two varieties, and maybe coupled with their relatively high photosynthetic apparatus might have given rise to a high rate of photosynthesis in varieties Sky Jacket and Purple Voice, and this explains why these varieties also recorded high gain in fresh weight of the mother bulb at harvest. Similarly, Sky Jacket and Purple Voice also produced higher fresh weight and number of bulblets at harvest than the other varieties, whilst Jon Bos and Pink Pearl produced the least gain in weight at harvest due to their low chlorophyll content and photosynthetic apparatus.

In terms of flower quality, varieties Fondant and Blue Jacket produced the highest inflorescence height, inflorescence length and number of florets, but they recorded lower vegetative growth and bulb yield at harvest as compared to Sky Jacket. The low bulb yield of varieties Fondant and Blue Jacket could be a result of two causes: the first being the result of their relatively low photosynthetic apparatus and chlorophyll concentration as compared to Sky Jackets and Purple, which might have resulted in a low rate of photosynthesis and hence dry matter accumulation in the bulbs of these varieties. Alternatively, assimilates from current photosynthesis and/or reserves stored in the bulb scales of these varieties prior to planting or from current photosynthesis might have been diverted to the inflorescence (Die *et al*., 1970) for the development of this organ (flowers) instead of the resources being used for vegetative growth. In flower bulbs, like many other geophytes, the inflorescence is an important sink organ, and it depends on the reserves stored in the bulb for its growth and development. Thus the

depletion of carbohydrates reserves stored in the bulb scales led to the accumulation of carbohydrates, particularly starch in the flower at the early stages of growth even in the absence of photosynthesis. Similarly, the breakdown of stored reserves (starch) of the bulb scales, which resulted in high activities of α-amylase and starch phosphorylase during the early stages of development after planting of the hyacinth (Akazawa and Hara-Nishimura, 1985), also led to similar high activities of starch synthase and ADP glucose pyrophosphorylase in the flower (Chapter 3). Additionally, in Chapter 4, it was realised that scales excision led to either a reduction or complete inhibition of inflorescence formation. All these findings from the present study demonstrate how important the bulb scales, or the reserves stored in them, is to flower production. This suggests that once the reserves stored in the bulb scales are reduced as occurs when the bulb is damaged as a result of any stresses, or plant (bulb) - herbivore interaction, then, flowering will be seriously affected. Similarly, competition among organs for the reserves stored in the bulbs scales will reduce the amount of these reserves that will be available for flower development (Caldwell *et al*., 1987). The inflorescences in varieties Fondant and Blue Jacket might have had competitive advantage over the leaves in terms of acquisition of resources (carbohydrate reserves) for growth and thus more of the reserves were channelled into the flowers for the development of the inflorescence instead of leaf growth. Alternatively, the rate of photosynthesis in varieties Fondant and Blue Jacket might have been low, and hence dry matter accumulation in the bulbs might have been affected resulting in poor fresh weight of the mother bulb at harvest. In the light of these observations, growers should consider varieties Sky Jackets and Purple Voice during times of selection of varieties for planting when they aim at producing high bulb yield at harvest or if bulblets production is central to their objective of planting the flower bulb. However, varieties Fondant or Blue Jacket should be selected for planting because they have the genetic potential of producing high flower quality for use particularly in the perfumery industry, since hyacinth flowers are used in the manufacturing and processing of oils and perfumes (Gender, 1994; Usher, 1974). The greater the quantity of flowers produced, the higher the amount of oils and perfumes that will be extracted. Therefore, varieties Fondant and Blue Jacket must be considered by growers who wish to produce these flower bulbs for their perfumery uses. However, in general, flower bulbs are planted for their uses as cut flowers, potted plants or as landscaping plants. For indoor decorations, varieties such as Pink Pearl and Jon Bos can

be considered because they are small and portable, and can easily be handled as potted plants.

7.8 Hyacinth starch metabolism genes

Results of the blast search indicated that, in 2008, the sequences generated for ADP glucose pyrophosphorylase, starch phosphorylase, starch branching enzyme and starch synthase from hyacinth did not match with anything. Schmalenberger *et al*. (2001) reported that this observation occurs as a result of the primers hybridising to other genomic sequences. However, in 2009/2010, the sequences produced for starch phosphorylase and starch synthase from hyacinth were similar to the sequences of these enzymes from a number of plants. The starch phosphorylase sequence produced in this study was similar to the sequences of this enzyme from *Ipomoea batata*, *Ricinus communis, Arabidopsis thaliana, Triticum aestivum Zea mays* and some few other plants from which the primers were designed, but the sequence from *Ipomoea batata* was the closest in terms of similarity.

The starch synthase sequence from hyacinth was also similar to sequences of this enzyme from a number of plant species such as *Sorghum bicolor, Aegilops tauschii, Hordeum vulgare, Zea mays, Triticum aestivum, Oryza sativa* and *Amaranthus cruentus***,** but that from *Sorghum bicolor* gave the highest identity. This observation agrees with Salehuzzaman *et al*. (1993) who also reported that the sequence generated from starch synthase clone constructed from cDNA library from cassava tuber had 74% identity with potato starch synthase, but the percentage identity from other plant species varied from 60 to 72 %.

The two sequences generated from this work could be used to make specific primers with a view to isolating more of the gene from hyacinth DNA. They could be used to probe a hyacinth genomic library to get the whole gene (Zhang *et al*., 2002; Lutz, 2003). These sequences may be used to design new primers which are specific to hyacinth and this will help any studies in the future on full characterisation of these genes. This will be achieved by making and probing genomic libraries, and isolating clones from cDNA libraries. There will also be the need to investigate the expression of these genes by using specific probes for RT-PCR.

7.9 Conclusions and recommendations

The results of this thesis highlight the biochemistry of carbohydrate metabolism, particularly in relationship to the regulation of partitioning and metabolism of starch in hyacinth, and the general aspects of growth and physiology of flower bulbs, with special reference to hyacinth and the lily. Results indicated that, starch was the major storage carbohydrate in the bulbs of the two species. In general, starch and biomass contents of the bulb scales decreased whilst those of the newly formed organs increased after planting. Following the depletion of reserves in the bulb scales, the flower accumulated the highest amount of starch and biomass in hyacinth, whilst in the case of the lily; it was the stem and roots that accumulated the highest amount of these quantities. In hyacinth, starch degradation was more of amylolysis than phosphorolysis, and the activities of the starch degrading enzymes were highest at the time of sprouting. The distribution of enzymes involved in starch degradation in the bulb scales closely mimicked the pattern of starch degradation in these organs whilst the accumulation of starch and biomass of the newly formed organs also followed the accumulation of the polysaccharide in the newly formed organs. ADP glucose pyrophosphorylase and starch synthase were equally responsible for the accumulation of starch in the flower, as well as the newly formed leaves, but these enzymes were less responsible for starch accumulation in the stem and roots. The sequences produced in this study for starch phosphorylase and starch synthase can be used in making specific primers and this will enable the isolation of more of the genes from hyacinth DNA. They can be used to probe the genomic library of hyacinth to get the whole gene and this will allow the full characterisation of these genes.

Experiments on the response of the flower bulbs to herbivory, and drought stress also showed that, plants produced from peeled bulbs prior to planting took longer to sprout, and recorded reductions in vegetative growth and flower quality as compared to the unpeeled control. In hyacinth, peeling of bulb before planting also resulted in poor bulb yield at harvest, but in the lily, the treatment, particularly that involving complete scale removal, produced plants that recorded a higher gain in fresh weight of bulb at harvest. In general, plants produced from peeled bulbs behaved just like those planted from small bulbs because vegetative growth and flowering were reduced relative to those from the unpeeled. Thus it seems that in flower bulbs, flowering is intimately linked to the reserves of the planted bulb, rather than the leaf growth produced. Also, hyacinth

plants whose leaves were defoliated did not compensate for the leaf loss because growth was always reduced but flowering was unaffected. However, in the case of the lily, plants responded positively to complete shoots herbivory especially when the damage was exerted at the beginning of their growth. Hyacinth and the lily survived dry periods as bulbs but both plants were very susceptible to drought stress in the growing phase of their life cycles. Therefore any adaptations bulbs possess to survive drought must be invested in the dormant bulb.

Studies on the response of these flower bulbs to some agronomic practices revealed that, vegetative growth, flower quality and bulb yield at harvest generally increased with increasing bulb size at planting. But in hyacinth, values of growth, flower quality and bulb yield parameters were similar after the 50 g bulb size. Plants produced from large bulbs grew faster, and completed their life cycle earlier than those planted from small bulbs. Moreso, deep planting (15-20 cm) of bulbs resulted in a delay in emergence, and this affected growth, flower yield and bulb production of these plants as compared to those of shallow planting. Bulbs responded to shallow planting by "pulling" themselves down into deeper ground. The shallower the depth of planting, the greater the movement made by the bulbs at the end of the season. For bulbs of hyacinth and the lily, growth, flower quality, bulb and bulblets yield were optimum when bulbs were planted at the 5 cm depth. Therefore, in case of planting in pots, this planting depth should be considered by growers for increased yield and quality products of these flowers bulbs.

In general, plants that received nutrients application from either $(NH_4)_2SO_4$ or Na_2HPO_4 performed better than the control, and the magnitude of growth and yield parameters measured increased with increases in the rate of application of the nutrients. The application of $(NH_4)_2SO_4$ at the 90 mM rate produced the highest growth, chlorophyll concentration, photosynthetic rates and bulb yield in both species. However, in terms of flower quality, it was the application of Na_2HPO_4 at the rate 60-90 mM that produced the best in hyacinth, but in the lily, plants that received nutrients from $(NH_4)_2SO_4$ at the 90 mM recorded the best flower quality. However, the present study did not investigate into the combined effects of N and P on growth and productivity of these bulbs. It is expected that future research on this area of study should address that.

On hyacinth varietal evaluation, variety Sky Jackets, followed by Purple Voice produced the highest vegetative growth, mother bulb fresh weight and bulblets yield at harvest whilst Jon Bos and Pink Pearl produced the least values of growth and yield parameters. Fondant and Blue Jacket recorded the highest flower quality because they produced the highest inflorescence height and length, and inflorescence diameter. Therefore, varieties Fondant and Blue Jacket must be considered by growers who wish to produce these flower bulbs for their uses in the perfumery industry, but for indoor decorations or as landscaping plants, varieties such as Pink Pearl and Jon Bos can be considered because they have small leaf canopy architecture, and can easily be handled as potted plants.

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